Universidad de Alcalá
Departamento de Ciencias de la Vida
Unidad Docente Ecología

Ecological impacts of exotic invasive trees on the structure and functioning of fluvial and riparian ecosystems

Memoria presentada para optar al grado de Doctora por la Universidad de Alcalá

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"Ecología, Conservación y Restauración de Ecosistemas"

(D330)

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hace constar:

que el trabajo descrito en la presente memoria, titulado "***Ecological impacts of exotic invasive trees on the structure and functioning of fluvial and riparian ecosystems***", ha sido realizado bajo su dirección por Silvia Medina Villar en la Unidad Docente de Ecología del Departamento de Ciencias de la Vida de la Universidad de Alcalá, dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D330), reuniendo todos los requisitos necesarios para su aprobación como Tesis Doctoral.

Alcalá de Henares, 24 de Mayo de 2016

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Mª Esther Pérez Corona, Profesora Titular del Departamento de Ecología de la Universidad Complutense de Madrid y co-directora de esta Tesis Doctoral,

hace constar:

que el trabajo descrito en la presente memoria, titulado "***Ecological impacts of exotic invasive trees on the structure and functioning of fluvial and riparian ecosystems***", ha sido realizado bajo su dirección por Silvia Medina Villar en la Unidad Docente de Ecología del Departamento de Ciencias de la Vida de la Universidad de Alcalá, dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D330), reuniendo todos los requisitos necesarios para su aprobación como Tesis Doctoral.

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hace constar:

que el trabajo descrito en la presente memoria, titulado "*Ecological impacts of exotic invasive trees on the structure and functioning of fluvial and riparian ecosystems*", ha sido realizado dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D330), reuniendo todos los requisitos necesarios para su aprobación como Tesis Doctoral, por acuerdo del Consejo de Departamento celebrado el día 20 de Mayo de 2016.

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Dr. Michel Heykoop Fung-a-you

La Tesis Doctoral que se presenta se desarrolla amparada con una beca FPI del Ministerio de Economía y Competitividad (BES-2011-048379) y todos sus objetivos se desarrollan en el marco del proyecto POII10-0179-4700 "*Evaluación integral de los impactos de los árboles exóticos invasores sobre los ecosistemas fluviales y de ribera de Castilla-la-Mancha*", el proyecto CGL2010-16388/BOS "*Evaluación del riesgo invasor de árboles exóticos: patrones de distribución, éxito invasor e impacto en los ecosistemas (INVARISK)*" y los Programas REMEDINAL-2 S2009/AMB-1783 y REMEDINAL-3 S2013/MAE-2719 cofinanciados por la Comunidad de Madrid y la Unión Europea a través de fondos FEDER y FSE.

Cover photography: Remaining leaf and rachises of an individual of *Ailanthus altissima* near Chiloeches village, Guadalajara, Spain.

By: Luciano Pataro

A mis padres,
Sandra,
Diego,
Chloe y
Luciano

.

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La felicidad solo es real cuando es compartida
(*Happiness only real when shared*)
Christopher McCandless

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Thank you!

Fragmento de "Balada de Coimbra"

Ai céus de Setembro-Outubro,
painéis de sonho e loucura,
rasgando a toda a lonjura
Cenários de arrepiar,
que é de esses olhos de abismo
que à tarde a vós se elevam,
por longe andavam, voltavam,
Vos devolviam no olhar...?

Sem saber o que buscavam,
Que haviam de ir encontrar?

Chegam da Baixa até Celas
os ais dos sinos na bruma.
Se o céu tem tantas estrelas,
Importa lá cair uma!

No Choupal quis fazer versos,
Olhei as folhas do chão.
Deus sabe os sonhos dispersos
Que o vento leva na mão!

Ai águas do meu Mondego
Que entre choupais murmurando
Se me esquivais, nesse brando
Sempre ir andando até mar,
Que é das mãos roxas de febre
Que em vós se desalteravam,
E entre as folhas que boiavam
Se deixavam arrastar...?

Sem saber o que buscavam,
Que haviam de ir encontrar?

A Santa Cruz, um por um,
Dos troncos fui despedir-me.
Não tenho amigo nenhum
Que me haja sido tão firme...

José Régio

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La presente Tesis Doctoral está escrita en formato bilingüe para aspirar a la Mención Internacional

This current PhD Thesis is written in a bilingual format to aspirate to the International Mention

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Resumen

Abstract

The true sign of intelligence is not knowledge but imagination

Albert Einstein

Fotografía: Dosel de la especie exótica invasora *Ailanthus altissima* en un bosquete de ribera cerca de la población de Chiloeches (Guadalajara, España).
Por: Luciano Pataro

Photography: Canopy of the exotic invasive species *Ailanthus altissima* in a riparian forest near Chiloeches village (Guadalajara, Spain).
By: Luciano Pataro

Resumen

Las especies exóticas invasoras (EEI) son consideradas como una de las principales amenazas para la biodiversidad en el mundo, así como uno de los principales componentes del cambio global. Además, los impactos tanto ecológicos como económicos que producen las EEI suscitan una gran preocupación a nivel mundial, motivando la aparición de leyes que regulan su introducción en nuevas áreas y su gestión. El conocimiento de los impactos ecológicos de las EEI permite tomar decisiones encaminadas a priorizar la gestión de las especies más dañinas. Además, la información procedente de los estudios de impactos de las EEI es relevante para los análisis de riesgo, los cuales constituyen una herramienta fundamental para la prevención de la entrada de especies exóticas en áreas donde son potencialmente invasoras. A pesar de que los impactos ecológicos dependen enormemente del contexto (de la especie invasora, de la comunidad de especies nativas y el ecosistema invadido), los estudios sobre impactos de las EEI están actualmente sesgados a un número determinado de especies y ecosistemas. Por tanto, son necesarios más casos de estudio que permitan un mejor conocimiento de los impactos de las EEI en los ecosistemas que invaden.

Los ecosistemas de ribera son especialmente vulnerables a la invasión por especies exóticas. Esto es debido, en parte, a la elevada degradación que han sufrido estos ecosistemas a causa de diferentes actividades humanas. Por ejemplo, la canalización de los ríos, supone la destrucción de la vegetación nativa y deja espacios vacíos y disponibles para el establecimiento de las EEI. La presencia de EEI en las riberas puede tener consecuencias ecológicas tanto en el ecosistema terrestre como acuático ya que las riberas suponen zonas de transición que conectan ambos ecosistemas. *Ailanthus altissima* (Mill.) Swingle y *Robinia pseudoacacia* L. son dos árboles exóticos que invaden ecosistemas de ribera del Centro Peninsular. Sin embargo, el conocimiento de sus impactos ecológicos tanto en los ecosistemas de ribera y como en los fluviales es escaso.

El principal objetivo de esta tesis es evaluar los impactos ecológicos de las especies de árboles exóticos invasores, *A. altissima* y *R. pseudoacacia,* sobre propiedades bióticas y abióticas de los ecosistemas de ribera y fluviales desde un enfoque integrado.

En primer lugar, se evaluó si las EEI, *A. altissima* y *R. pseudoacacia,* alteraban la cantidad, contenido en nutrientes y la dinámica temporal de caída de la hojarasca producida en ecosistemas de ribera del Centro Peninsular (***Capítulo 2***). También se evaluó el impacto en propiedades del suelo, como son la concentración de nitrógeno (N), fósforo (P), materia orgánica (MO) y pH. Nuestros resultados mostraron que ni *A. altissima* ni *R. pseudoacacia* alteran la cantidad de hojarasca, pero sí su contenido en nutrientes y dinámica temporal de caída. Así, la hojarasca de los árboles invasores presentó mayores concentraciones de N y P que la del árbol nativo *Populus alba*, lo que probablemente determinó la mayor concentración de dichos nutrientes en el suelo de los bosques invadidos. Además, *A. altissima* y *R. pseudoacacia* produjeron picos adicionales de caída de hojarasca en primavera tardía y verano, los cuales no fueron observados en bosques dominados por las especies de árboles nativos, *Fraxinus angustifolia* Vahl. y *P. alba*.

El impacto de *A. altissima* y *R. pseudoacacia* en la comunidad de bacterias edáficas, en el pH, la concentración de N, P, MO y las tasas de mineralización de los nutrientes del suelo se estableció mediante dos aproximaciones: 1) un muestreo de suelo en bosques de ribera invadidos y no invadidos (éstos últimos dominados por la especie nativa *P. alba*) y 2) un experimento de invernadero donde se sembraron semillas de las EEI (*A. altissima* y *R. pseudoacacia*) y de la especie nativa (*P. alba*) en un suelo natural y se dejaron crecer durante seis meses (***Capítulo 3***). Tanto en campo como en el experimento de invernadero, respecto de los suelos de *P. alba,* los suelos de *R. pseudoacacia* presentaron mayor concentración de N mineral, mientras que los de *A. altissima* mostraron menor concentración de N orgánico. Las diferencias entre suelos invadidos y no

invadidos en otras variables, como las tasas de mineralización o la estructura de la comunidad de bacterias del suelo, aparecieron solo en los suelos del muestreo de campo, siendo las tasas de mineralización del N y la actividad fosfomonoesterasa (PME) del suelo mayores en los bosques invadidos por *R. pseudoacacia* y menores en los bosques invadidos por *A. altisssima* que en los bosques dominados por la especie nativa *P. alba*; La comunidad de bacterias del suelo en bosques invadidos por *A. altissima* fue claramente diferente a la de los bosques de *P. alba*. En conjunto, este estudio sugiere que *A. altissima* y *R. pseudoacacia*, pueden producir algunos impactos en etapas tempranas de establecimiento, pero necesitan más tiempo para producir otros impactos en las comunidades microbianas y en determinados procesos del ciclo de nutrientes.

En esta Tesis se evaluó también el efecto alelopático de extractos acuosos de hojas senescentes (de aquí en adelante "extractos") de especies de árboles exóticos invasores (*A. altissima* y *R. pseudoacacia*) y nativos (*F. angustifolia* y *P. alba*) sobre la germinación y el crecimiento radicular de especies de plantas del sotobosque (***Capítulo 4***). Se analizaron concentraciones realistas de los extractos y se consideraron dos tipos de substrato: papel de germinación y suelo natural, este último procedente de un ecosistema de ribera. Nuestros resultados mostraron que los extractos de las especies exóticas invasoras no afectaron más intensamente a las plantas del sotobosque que los de las nativas. No obstante, los extractos de *R. pseudoacacia* fueron los más efectivos sobre las especies del sotobosque, y la especie *Trifolium repens* fue la más sensible a los diferentes extractos. Finalmente, el suelo de ribera disminuyó claramente los efectos alelopáticos encontrados en papel de germinación, lo que sugiere que la alelopatía puede ser un mecanismo menos importante de interacción entre árboles y especies del sotobosque bajo condiciones naturales.

Por último, se investigó el impacto de la invasión de los ecosistemas de ribera por *A. altissima* y *R. pseudoacacia* sobre los ecosistemas fluviales (***Capítulo 5***). Específicamente, se compararon las tasas de descomposición y la

colonización fúngica y por macroinvertebrados de la hojarasca de los árboles exóticos invasores (*A. altissima* y *R. pseudoacacia*) y nativos (*F. angustifolia* y *P. alba*) en un río de cabecera del Centro Peninsular. La hojarasca exótica fue menos colonizada por hongos que la nativa, apoyando la *Hipótesis de las Nuevas Armas*. Igualmente, el efecto de los macroinvertebrados en la descomposición de la hojarasca fue menor en especies exóticas que en nativas. Sin embargo, las tasas de descomposición fueron determinadas principalmente por características específicas de la hojarasca, tales como la concentración de lignina, fibra ácido detergente, P y los ratios N:P y lignina:P, más que por su origen exótico o nativo. Por tanto, la descomposición de la hojarasca fue especie-específica, descomponiéndose la hojarasca de *A. altissima* más rápidamente y la de *R. pseudoacacia* más lentamente que la de las especies nativas.

Para concluir, esta Tesis Doctoral muestra que *A. altissima* y *R. pseudoacacia* pueden producir importantes impactos ecológicos en los ecosistemas de ribera y fluviales. Los impactos de estas especies fueron muchas veces de sentido contrario, y otras veces en el mismo sentido. Se puede resaltar, como consecuencias de la invasión de los ecosistemas de ribera por *R. pseudoacacia*, la producción de hojarasca rica en N y difícil de descomponer, el aumento en el suelo de la concentración de materia orgánica, N total y mineral y de las tasas de mineralización de nutrientes. Respecto de la invasión de ecosistemas de ribera por *A. altissima,* se puede destacar la producción de hojarasca fácil de descomponer, la disminución de las tasas de mineralización de nutrientes en el suelo y los cambios en la comunidad bacteriana del suelo. Ambas especies disminuyeron la colonización fúngica de la hojarasca en un ecosistema fluvial.

Palabras clave: *Ailanthus altissima*; ciclos de nutrientes; consecuencias ecológicas; ecosistemas de ribera; *Fraxinus angustifolia*; hojarasca; *Populus alba*; *Robinia pseudoacacia*

Abstract

Exotic invasive species (EIS) are considered as one of the main world biodiversity threats as well as one of the main drivers of the global change. Besides, the ecological and economic impacts produced by EIS have generated worldwide concern, which have motivated the emergence of legislation to regulate their introduction and management. The knowledge about the ecological impacts of the EIS helps decision-making guided to the prioritization of the most harmful species. In addition, the information from the studies about EIS impacts is relevant to risk analyses, which constitutes an essential tool for exotic species prevention in areas where they are potential invaders. Although ecological impacts greatly depend on the invasive species, the native community and the recipient ecosystem, studies about EIS impacts are biased to a certain number of species and ecosystems. Therefore, it is necessary more case studies allowing better knowledge of the EIS impacts on the ecosystems they invade.

Riparian ecosystems are especially vulnerable to the invasion by exotic species. It is in part due to the high level of degradation these ecosystems suffer as a consequence of several human activities. For instance, river channelization implies the destruction of native vegetation, which creates gaps to the establishment of EIS. The occurrence of EIS in riparian areas can have consequences in both terrestrial and aquatic ecosystems due to riparian areas are transitional zones connecting both ecosystems. *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. are two exotic trees invading riparian ecosystems of the center of the Iberian Peninsula. However, the knowledge about their ecological impacts in both fluvial and riparian ecosystems is scarce. The aim of this Thesis was the evaluation of the ecological impacts of the exotic invasive trees, *A. altissima* and *R. pseudoacacia,* on the biotic and abiotic properties of riparian and fluvial ecosystems following an integrated approach.

Firstly, we evaluated if the EIS, *A. altissima* and *R. pseudoacacia,* altered the quantity, quality and temporal dynamic of litter produced in riparian ecosystems (***Chapter 2***). We also analyzed the impact on soil properties such as the concentration of nitrogen (N), phosphorus (P), organic matter (OM), and pH. Our results showed that neither *A. altissima* nor *R. pseudoacacia* altered the quantity of litter but both species altered the quality of litter (i.e. nutrient concentration) and the temporal dynamic of litterfall. Thus, litter from invasive trees showed greater concentrations of N and P than litter from the native tree *Populus alba* L., which probably determined greater concentration of the mentioned nutrients in the soil of the invaded forests. Besides, *A. altissima* and *R. pseudoacacia* produced additional litter peaks in late-spring and summer, which lacked in forests dominated by the native tree species *Fraxinus angustifolia* Vahl. and *P. alba*.

We investigated the impact of *A. altissima* and *R. pseudoacacia* on the soil bacterial community, soil properties (pH, concentration of N, P and OM) and net mineralization rates of N and P by two approach: 1) a field soil sampling in invaded and non-invaded (dominated by the native *P. alba*) riparian forests, and 2) a greenhouse experiment where EIS (*A. altissima* and *R. pseudoacacia*) and the native species (*P. alba*) were grown from seeds in a natural soil during six months (***Chapter 3***). Both in the field and in the greenhouse experiment, the soils from *R. pseudoacacia* showed greater concentration of N mineral while soils from *A. altissima* showed lower concentration of organic N than *P. alba* soils. Differences between invaded and non invaded soils in other variables, such as the nutrient mineralization rates or the structure of soil bacterial community, appeared only in soils from the field sampling. The net N mineralization rate and the phosphomonoesterase activity were greater in soils from forests invaded by *R. pseudoacacia* and lower in soils from forests invaded by *A. altisssima* than in *P. alba* soils; The soil bacterial community in forests invaded by *A. altisssima* was clearly different as that in forests dominated by *P. alba.* Altogether, this study suggests that *A. altissima* and *R. pseudoacacia* can produce

certain impacts in early stages of establishment, but need more time of invasion to produce other impacts in microbial communities and nutrient cycling processes.

The allelopathic effects of the aqueous leaf litter extracts (hereinafter "extracts") from exotic invasive (*A. altissima* and *R. pseudoacacia*) and native (*F. angustifolia* and *P. alba*) tree species on the germination and radicle growth of several under-canopy plant species were also evaluated in this Thesis (***Chapter 4***). Realistic concentrations of the extracts were analyzed in two types of substratum: germination paper and natural soil from a riparian ecosystem. Our results showed that the extracts of the EIS did not affect the under-canopy plants more than those from the natives. Nonetheless, *R. pseudoacacia* extracts were the most effective against the under-canopy species and *Trifolium repens* was the most sensitive species to the extracts. Finally, the riparian soil clearly decreased the allelopathic effects found in germination paper, which suggests that allelopathy can be a less important mechanism of interaction among trees and under-canopy species under natural conditions.

Lastly, the impact of riparian ecosystem invasion by *A. altissima* and *R. pseudoacacia* on fluvial ecosystems was investigated (***Chapter 5***). Specifically, we compared the decomposition rate and the colonization by fungi and macroinvertebrates between leaf litter from the exotic (*A. altissima* and *R. pseudoacacia*) and native (*F. angustifolia* and *P. alba*) trees in a headwater stream of the center of Iberian Peninsula. The exotic leaf litter was less colonized by fungi than the native, according to the *Novel Weapons Hypothesis*. Similarly, the effect of macroinvertebrates on leaf litter decomposition was lower in exotic than in native species. However, litter decomposition rates were better determined by the specific leaf litter characteristics, such as the concentration of lignin, acid detergent fiber, P and the N:P and lignin:P ratios than by the origin of the litter (exotic or native). Therefore, leaf litter decomposition was species-

specific: leaf litter from *A. altissima* faster and from *R. pseudoacacia* slower than that from the native species.

It is concluded that *A. altissima* and *R. pseudoacacia* can produce significant ecological impacts on riparian and fluvial ecosystems. The direction of these impacts was often different but sometimes the same. From our results, we can highlight that *R. pseudoacacia* causes 1) a production of nutrient-rich litter, hard to decompose, 2) an increase of the concentration of soil organic matter, total and mineral N and 3) A rise in the nutrient mineralization rates in the soil. Regarding the riparian invasion by *A. altissima,* we can highlight: 1) the the production of leaf litter easy to decompose, 2) the decrease of soil nutrient mineralization rates and 3) the modification of soil bacterial communities. In common, both species decreased the fungal colonization of leaf litter in a fluvial ecosystem.

Keywords: *Ailanthus altissima*; ecological consequences; floodplain ecosystems; *Fraxinus angustifolia*; litter; nutrient cycling; *Populus alba*; *Robinia pseudoacacia*

Capítulo 1

Introducción general

Lo más fácil es reaccionar. Lo segundo más fácil es responder. Pero lo más difícil es empezar.

Seth Godin

Fotografía: La especie exótica invasora, *Ailanthus altissima,* creciendo junto al *Edificio do Botánico* (Coimbra, Portugal)
Por: Silvia Medina Villar

Photography: The exotic invasive species, *Ailanthus altissima,* growing next to the *Edificio do Botánico* (Coimbra, Portugal)
By: Silvia Medina Villar

1. Introducción a las especies exóticas invasoras

1.1. Especies exóticas

Las especies exóticas (EE), también llamadas alóctonas, son aquellas especies introducidas fuera de su área de distribución natural por el ser humano, ya sea de forma accidental o intencionada (Richardson et al. 2000). Con la globalización, ha aumentado el transporte de personas y de mercancías en el tiempo y en el espacio y, con ello, la introducción de especies exóticas en nuevas áreas. El movimiento actual de especies debido a la acción del ser humano afecta a un gran número de regiones, por lo que no es comparable a la dispersión natural de las especies producida en épocas geológicas anteriores (Ricciardi 2007). Con respecto a las especies de plantas, su introducción a lo largo de la historia en áreas fuera de su rango nativo ha respondido a fines de restauración (ej. estabilización de taludes), agrícolas, forestales, horticulturales u ornamentales, siendo estos dos últimos las principales vías de entrada de EE en nuevas áreas (García-Berthou et al. 2008; Pyšek et al. 2009; Richardson y Rejmanek 2011).

En función de la escala temporal, la consideración de una especie como exótica no es tan clara (Falk-Petersen et al. 2006; Valery et al. 2008). Por ejemplo, ¿puede considerarse exótica una especie que se extinguió en una región antes o durante la última glaciación (aprox. 100.000-12000 años AC) y que ha sido después reintroducida por el ser humano? (Pyšek 1995). En respuesta a esta cuestión, se ha sugerido que las especies extintas y reintroducidas post-glaciación deben considerarse exóticas, ya que no han evolucionado con las condiciones climáticas actuales, las cuales son diferentes a las existentes antes de la última glaciación (Kowarik 1995; Pyšek 1995). Atendiendo a una escala temporal menor, las especies introducidas por el hombre antes del Neolítico (aprox. 5000-6000 años AC) no suelen considerarse como exóticas ya que en ese momento la dispersión de especies por parte del ser humano era equivalente a la de otros animales (Webb 1985;

Pyšek 1995). No obstante, las especies introducidas antes del año 1500 generalmente no presentan carácter invasivo y no causan problemas ecológicos, por lo que no suelen ser objeto de estudio en invasiones biológicas, considerándose especies equivalentes a las nativas (Pyšek 1995; Richardson et al. 2000).

1.2. Especies exóticas invasoras

De forma general, puede definirse especie exótica invasora (EEI) como un organismo que se ha establecido en una nueva área geográfica y está expandiendo su rango de distribución (Falk-Petersen et al. 2006). No obstante, el proceso de invasión biológica, desde que una especie es introducida hasta que se convierte en invasora, no es tan frecuente debido a que la especie debe superar una serie de barreras geográficas, ambientales, reproductivas y dispersivas (Richardson et al. 2000). Siguiendo la "regla de los dieces", alrededor del 10 % de las especies introducidas se naturalizan, es decir, se reproducen y mantienen poblaciones sin intervención directa del ser humano (Williamson et al. 1996; Richardson et al. 2000). A su vez, aproximadamente un 10 % de las especies naturalizadas se convierten en invasoras, es decir, son capaces de expandirse en un área considerable en poco tiempo (Williamson et al. 1996; Richardson et al. 2000).

La consideración de EEI no es siempre tan clara, siendo su definición motivo de debate (Falk-Petersen et al. 2006; Valery et al. 2008). Algunos autores han expuesto que una especie nativa también puede dispersarse grandes distancias cuando coloniza naturalmente nuevas áreas (Davis y Thompson 2000). En este sentido, Richardson y colaboradores (2000) establecieron una escala espacio-temporal para considerar una planta exótica como invasora. Así, una planta exótica se considera como invasora si ha podido dispersarse más de 100 metros en menos de 50 años, en el caso de que se reproduzca sexualmente, y 6 metros en menos de 30 años si se reproduce asexualmente (Richardson et al. 2000). Sin embargo, esta escala

espacio-temporal para definir una planta como invasora es imprecisa, ya que no tiene en cuenta otros aspectos de la biología de las especies, como su tamaño o su tipo de dispersión (zoocora, anemocora, etc.).

Otros autores establecen que para considerar una especie exótica como invasora, además de dispersarse en una nueva área, debe producir algún impacto significativo en los ecosistemas y/o comunidades (Cronk y Fuller 1995; Mack et al. 2000; Davis y Thompson 2000). De hecho, el impacto ha sido sugerido como la última etapa del proceso de invasión (Figura 1). Sin embargo, siguiendo el razonamiento de Valery y colaboradores (2008), la definición de un fenómeno debe residir únicamente en criterios relativos a su "esencia" y no a sus efectos, que son circunstanciales y variables. Además, otras de las razones para no incluir el impacto en la definición de EEI responden a la dificultad para establecer qué se considera como impacto significativo, cuáles son sus valores umbrales, así como el hecho de que una especie no invasora también pueda causar impactos (Parker et al. 1999; Richardson et al. 2000; Daehler 2001; Valery et al. 2008). Consiguientemente, tanto especies nativas, como EE o EEI se denominan "pestes" (o "malas hierbas" en el caso de plantas) cuando producen daños sobre las actividades humanas y "transformadores" o "ingenieros de ecosistemas" cuando producen profundos impactos en los ecosistemas (Richardson et al. 2000; Pimentel 2002; Falk-Petersen et al. 2006).

1.3. Impactos de las especies exóticas invasoras

A pesar del debate sobre la inclusión o no del impacto en la definición de EEI, una de las razones más importantes que motiva el estudio de las EEI es su capacidad de producir impactos, tanto ecológicos (sobre las especies y los ecosistemas) como económicos o sociales (Pimentel 2002; Charles y Dukes 2007; Simberloff et al. 2013). De hecho, las EEI son consideradas como una de las principales amenazas para la biodiversidad en el mundo (Cronk y Fuller 1995; McGeoch et al. 2010), así como uno de los principales componentes del

denominado cambio global (Vitousek et al. 1997), cifrándose sus costes económicos en miles de millones de dólares cada año (Pimentel 2002). Al mismo tiempo, existen EEI de todos los grupos taxonómicos que afectan a los servicios de los ecosistemas, es decir a los beneficios que reportan los ecosistemas naturales al ser humano (Charles y Dukes 2007; Pejchar y Mooney 2009; Vilà et al. 2010). Por todo lo anterior, Larson (2007) sugiere que no considerar el impacto como una característica de las EEI es inconsistente con los valores de conservación de la biodiversidad, ya que puede dar lugar a la no actuación frente a las invasiones.

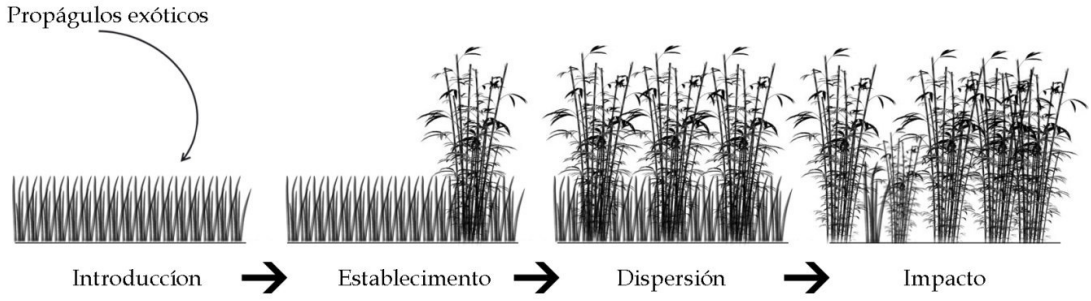


Figura 1. Proceso de invasión biológica (Modificado de **Levine 2008**).

Esta Tesis se centra en los impactos ecológicos, que pueden definirse como cualquier cambio medible y significativo en un patrón, propiedad o proceso de un ecosistema (Pyšek et al. 2012; Ricciardi et al. 2013; Jeschke et al. 2014). Según esta definición: 1) toda EE produce un impacto por el simple hecho de estar integrada en el sistema, 2) los impactos pueden variar en dirección (ser positivos o negativos) y magnitud, y 3) los impactos pueden ser comparados a través del tiempo y del espacio (Ricciardi et al. 2013). Por último, los impactos ecológicos pueden medirse a nivel de región, ecosistema, comunidad, especie y organismo (Ricciardi et al. 2013).

1.4. Marco legislativo

Los impactos económicos y ambientales producidos por las EEI han generado preocupación a nivel mundial, siendo el motivo por el cual ha sido necesario regular su introducción, control y gestión. La primera iniciativa transcendental a nivel global que contempla las EEI fue el Convenio sobre la Diversidad Biológica (CDB), que fue creado por el Programa Ambiental de las Naciones Unidas (PNUMA) y presentado en Junio de 1992 en la Conferencia de las Naciones Unidas sobre el Medio Ambiente y el Desarrollo (Cumbre de la Tierra de Río de Janeiro). Forman parte del CDB 196 países entre los que se incluye España. El artículo 8h del CDB establece que cada parte, impedirá la introducción, controlará y erradicará las EE que amenacen ecosistemas, hábitats o especies.

En España, la introducción o liberación no autorizada de especies alóctonas perjudiciales para el equilibrio biológico figura como delito contra el medio ambiente (Ley orgánica 10/1995 del Código Penal). La Ley 42/2007 del Patrimonio Natural y de la Biodiversidad, prohíbe la introducción, posesión, transporte, tráfico y comercio de especies, subespecies o razas geográficas alóctonas, cuando éstas sean susceptibles de competir con las especies silvestres autóctonas, alterar su pureza genética o los equilibrios ecológicos. Esta Ley define las EEI como "*aquellas especies que constituyen, de hecho, o puedan llegar a constituir una amenaza grave para las especies autóctonas, los hábitats o los ecosistemas, la agronomía, o para los recursos económicos asociados al uso del patrimonio natural*" y se aplica a las especies del Catálogo español de EEI de 2013 (RD 630/2013), que deroga los anteriores de 2011 y 2007. La elaboración de éste y otros catálogos contemplan aquellas especies más perjudiciales para los ecosistemas o las actividades humanas. En ocasiones, intereses socioeconómicos y políticos determinan la no inclusión en los catálogos de determinadas EEI, dificultando las actuaciones de prevención y gestión de las mismas. Con todo, la inclusión de especies en los catálogos de EEI se rige principalmente por la información científico-técnica disponible.

Por ello, la escasez de información sobre los impactos de las EEI puede también implicar que algunas especies dañinas no se incluyan en los catálogos. De ahí la relevancia de los estudios científicos que evalúan los impactos de las EEI para la toma de decisiones sobre la elección de las EEI más dañinas para los ecosistemas naturales y la economía. Existen otros ejemplos de listas, catálogos y atlas tanto de EE como de EEI a nivel mundial (GISD, *Global Invasive Species Database*, http://www.issg.org/database/welcome/; Richardson y Rejmanek 2011), europeo (DAISIE, *Delivering Alien Invasive Species Inventories for Europe*, http://www.europe-aliens.org/; NOBANIS, *European Network on Invasive Alien Species*, https://www.nobanis.org/), nacional (Pyšek et al. 2002; Sanz-Elorza et al. 2004; GEIB 2006) o autonómico (Campos y Herrera 2009; Sanz-Elorza et al. 2008, 2009, 2011).

Las labores de gestión de EEI requieren un coste muy elevado, sobre todo cuanto mayor es el tiempo desde la introducción de la especie (Richardson y Rejmanek 2011; Hulme 2012). En consecuencia, los estudios que permitan conocer los impactos de las EEI son de gran relevancia para una gestión eficaz, centrada en las especies más dañinas (Hulme et al. 2013). También, el conocimiento de las amenazas producidas por las EEI es una información necesaria para la realización de los análisis de riesgo de determinadas especies con potencial invasor. La estimación del riesgo de una especie se basa en que sus consecuencias detectadas en una región en la que es invasora podrían producirse también en otras regiones susceptibles de ser invadidas. Así, la identificación de los impactos producidos por las EEI permite prevenir la introducción de especies de elevado riesgo, que serían aquellas con alta probabilidad de establecerse en un determinado área y causar impactos (Capdevila et al. 2006)

1.5. Causas de la invasión por especies exóticas invasoras

Además de conocer los impactos que producen las EEI, una de las cuestiones que trata de resolver la ecología de las invasiones biológicas es determinar por qué algunas EE invaden ciertos ecosistemas y otras no (Mack et al. 2000; Zedler y Kercher 2004). Numerosas hipótesis han sido formuladas para explicar las causas de la invasión por determinadas EE (Mack et al. 2000; Catford et al. 2009). Estas hipótesis pueden agruparse en función de si se refieren a las características de las EEI o del ecosistema receptor (Barney et al. 2008; Catford et al. 2009). La *Hipótesis de la Invasora Ideal* recoge la idea de que existen ciertas características de las EEI que les confieren ventajas competitivas y por tanto facilitan el proceso de invasión (Elton 1958; Rejmanek y Richardson 1996; Van Kleunen et al. 2010). Algunas de estas características son la elevada tasa de crecimiento, capacidad fotosintética, plasticidad fenotípica, tolerancia al estrés (herbivoría, escasez de nutrientes o de agua) y eficacia reproductiva y dispersiva (fertilidad, presencia de reproducción tanto asexual como sexual, floración más temprana o durante más tiempo, germinabilidad de semillas, etc.) (Sanz-Elorza et al. 2004; Godoy et al. 2008; Van Kleunen et al. 2010; Godoy et al. 2011). La *Hipótesis de las Nuevas Armas* expone que las EEI presentan compuestos que son "nuevos" para las especies nativas (de plantas o microorganismos), a las cuales afectan enormemente. Esto se debe a que las especies nativas no han evolucionado con las EEI y, por tanto, no están adaptadas a soportar los nuevos compuestos o no han tenido tiempo de desarrollar defensas contra ellos (Callaway y Ridenour 2004). Por otro lado, la ausencia o el menor número de enemigos naturales (patógenos, depredadores, herbívoros) en el ecosistema invadido también pueden permitir a las EEI competir mejor frente a las especies nativas (*Hipótesis de escape de los enemigos naturales,* Keane y Crawley 2002*; Hipótesis de aumento de habilidad competitiva,* Blossey y Notzold 1995).

Las hipótesis que tienen que ver con las características del ecosistema receptor establecen ecosistemas más susceptibles a la invasión, como son

aquellos que presentan nichos vacíos (*Hipótesis del Nicho Vacío*, MacArthur 1970). Además, los hábitats más heterogéneos presentan mayor disponibilidad de nichos y, por tanto una mayor probabilidad de ser invadidos por diferentes EE (Melbourne et al. 2007). También, los ecosistemas más perturbados presentan una mayor disponibilidad de recursos, lo que favorece la invasión de especies de plantas, sobretodo ruderales (*Hipótesis de la Perturbación*, Davis y Thompson 2000). Asimismo, una elevada frecuencia de introducciones (número de eventos y en grandes cantidades) de una especie en un ecosistema aumenta su probabilidad de invasión (*Hipótesis de elevada presión de propágulos,* Lonsdale 1999). En cuanto a la riqueza de especies nativas en el ecosistema receptor no está tan claro si favorece o dificulta la invasión del ecosistema (Barney et al. 2008).

Algunas interacciones entre la EEI y el ecosistema receptor pueden favorecer futuras invasiones, produciéndose una reacción en cadena de la invasión (del inglés *Invasional Meltdown Hypothesis,* Simberloff y Von Holle 1999). Por ejemplo, la modificación de algunas características del ecosistema, la interferencia con asociaciones mutualistas nativas o el establecimiento de asociaciones mutualistas nuevas o más efectivas entre la especie invasora y los microorganismos nativos, pueden facilitar la invasión por EE (Wardle et al. 1994; Stinson et al. 2006; Niu et al. 2007).

2. Las plantas exóticas invasoras

El 0.5% de las especies de árboles y arbustos en el mundo son exóticas invasoras (Richardson y Rejmanek 2011). Además, en Europa y España, aproximadamente la mitad de las especies exóticas (EE) registradas pertenecen a plantas terrestres (1692 y 856 especies, respectivamente) (DAISIE 2009). También, las plantas terrestres son uno de los dos grupos taxonómicos que presentan más EE causando impactos en los servicios de los ecosistemas en Europa (Vilà et al. 2010). En España, 123 plantas exóticas (10-14 % de la flora española) están naturalizadas y causando daños ecológicos,

incluso compitiendo con decenas de especies autóctonas amenazadas (Dana et al. 2003; Sanz-Elorza et al. 2004). A pesar de que las plantas son el grupo taxonómico más investigado a nivel mundial en el área de las invasiones biológicas (Pyšek et al. 2008), los estudios están sesgados a determinadas especies y regiones (Pyšek et al. 2008; Hulme et al. 2013). Son necesarios, por tanto, más trabajos de múltiples EEI que contemplen contextos particulares para tener una visión más clara de los procesos que generalmente intervienen en el éxito de invasión, las especies que producen mayores impactos y en qué ecosistemas (Hulme et al. 2013). La enorme dependencia del contexto en los estudios de invasiones biológicas y en particular en la producción de impactos dificulta hacer generalizaciones, lo que resalta aun más la necesidad de analizar más casos de estudio para cada especie invasora particular en los ecosistemas que invaden (Ricciardi et al. 2013). El mayor número de casos de estudio disponibles permitirá una mayor robustez de las técnicas de meta-análisis, ayudando a comprender mejor los impactos que producen las plantas invasoras (Castro-Díez et al. 2014a and b).

2.1. Impactos ecológicos de las plantas exóticas invasoras

Las plantas invasoras pueden producir impactos ecológicos a diferentes niveles: especie, comunidad o ecosistema (Ricciardi et al. 2013). A nivel de especie, las plantas invasoras pueden disminuir el crecimiento o la condición física (ej. supervivencia, fecundidad) de las especies nativas de plantas (Gould y Gorchov 2000; Gómez-Aparicio y Canham 2008a; Rudgers y Orr 2009) e invertebrados (Going y Dudley 2008). Asimismo, se ha observado la disminución de la pureza genética de plantas nativas por hibridación con plantas invasoras (Anttila et al. 1998). A nivel de comunidad, las plantas invasoras pueden afectar a la abundancia, riqueza, diversidad y composición específica o funcional de comunidades nativas de plantas (Vilà et al. 2006; Von Holle et al. 2006; Hejda et al. 2009; Motard et al. 2011), artrópodos (Heleno et al. 2008; Gutierrez et al. 2014; Motard et al. 2015) o

microorganismos (Kourtev et al. 2002; Hawkes et al. 2005; Wolfe y Klironomos 2005).

Los ecosistemas pueden verse afectados por las plantas invasoras debido a cambios en la frecuencia e intensidad de los fuegos (D´Antonio y Vitousek 1992) ó a alteraciones de los ciclos biogeoquímicos (Vitousek 1984; Kourtev et al. 1999; Ehrenfeld 2003; Blank 2008). Los efectos de las plantas invasoras pueden producirse sobre determinados procesos de los ciclos de nutrientes, así como sobre los organismos que los llevan a cabo (Kourtev et al. 1999; Albariño y Balseiro 2002; Hawkes et al. 2005). Especialmente, pueden verse afectados importantes procesos de los ciclos de nutrientes, como son la descomposición y mineralización de la materia orgánica (MO), los cuales permiten la producción de compuestos inorgánicos fácilmente asimilables por las plantas (ej. el nitrato, el amonio o el fósforo disponible) (Marschner y Rengel 2007; Lambers 2008) (Figura 2). Los invertebrados y microorganismos detritívoros, los cuales permiten la descomposición de la materia orgánica gruesa (> 1 mm), principalmente hojarasca, tanto en medio acuático (Allan y Castillo 2007) como terrestre (Marschner y Rengel 2007), también pueden verse afectados por las plantas invasoras (Figura 2). Del mismo modo, otros procesos principalmente controlados por microorganismos del suelo (bacterias y hongos), como son la producción y asimilación de formas minerales de N y P, la desnitrificación o la fijación del N atmosférico (Booth et al. 2005; Myrold y Posavatz 2007; Marschner y Rengel 2007) (Figura 2) han sido afectados por determinadas plantas invasoras (Wardle et al. 1994; Evans et al. 2001)

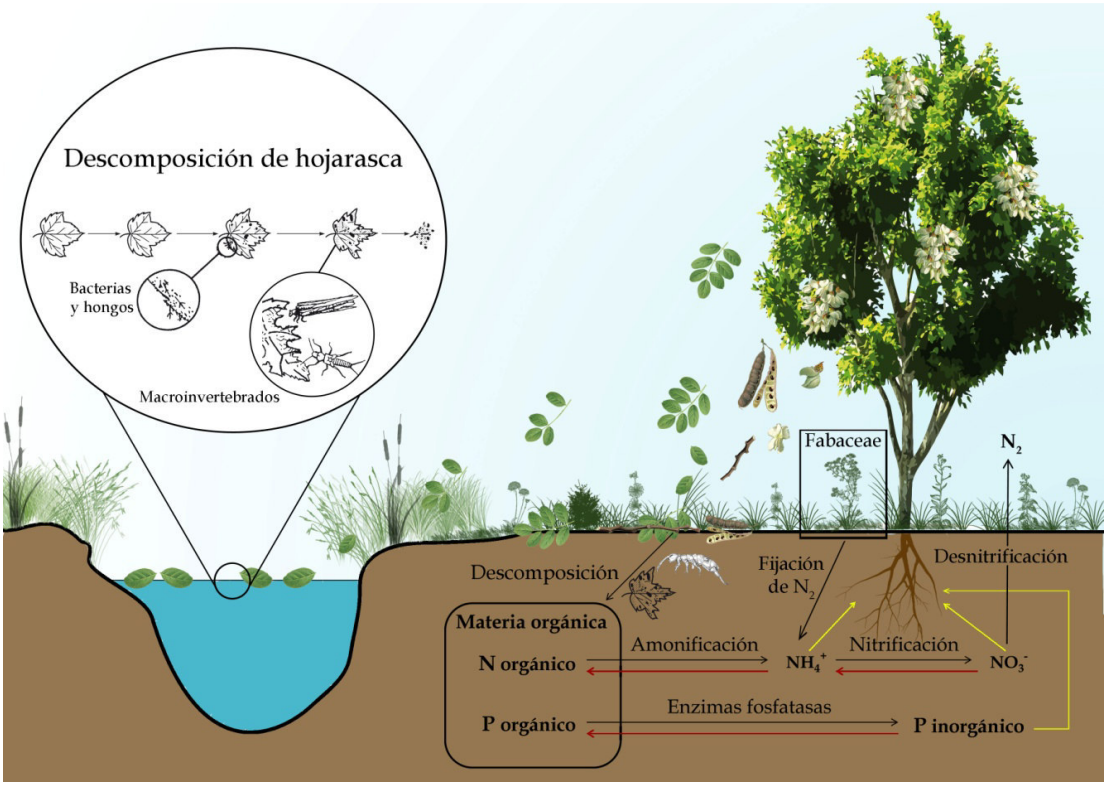


Figura 2. Esquema de diferentes procesos ecosistémicos que pueden verse afectados por la invasión de plantas exóticas. Las flechas en rojo indican procesos de asimilación (i. e. inmovilización) de compuestos inorgánicos (nitrato = NO_3^-; amonio = NH_4^+; fósforo inorgánico = P inorgánico) por los microorganismos del suelo. Las flechas amarillas indican procesos de asimilación de los compuestos inorgánicos por las plantas (Modificado de: Allan y Castillo 2007; Marschner y Rengel 2007).

De forma indirecta, los impactos producidos por las plantas invasoras en un nivel de organización (ecosistema, comunidad o especie) pueden afectar a otros niveles. Por ejemplo, los cambios en determinadas propiedades del suelo (nutrientes, salinidad, comunidad microbiana) pueden dificultar el crecimiento de determinadas plantas nativas (Vivrette y Muller 1977; Haubensak y Parker 2004; Niu et al. 2007). Del mismo modo, una menor diversidad de plantas nativas puede disminuir la riqueza de artrópodos herbívoros por reducción del alimento disponible (Litt et al. 2014). También, los cambios en las comunidades de bacterias, hongos y artrópodos del suelo ocasionados por plantas invasoras pueden alterar el ciclo de nutrientes (Hawkes et al. 2005; Wolfe y Klironomos 2005; Van der Putten et al. 2007). Así, se ha demostrado cómo el aumento en la abundancia

de bacterias nitrificantes por algunas herbáceas invasoras duplicó las tasas de nitrificación del suelo (Hawkes et al. 2005), mientras que la disminución en la abundancia de nematodos tras la invasión de plantas exóticas causó un aumento del pH y la MO del suelo (Wolfe y Klironomos 2005).

Además, las plantas invasoras pueden producir "efectos de legado", que son cambios que perduran durante años tras la eliminación de la planta invasora e incluso tras la restauración con plantas nativas (Malcolm et al. 2008; Elgersma et al. 2011, Von Holle et al. 2013). Por ejemplo, se han observado efectos de legado en la elevación de los niveles de N del suelo y en cambios en la estructura y actividad de la comunidad de microorganismos, los cuales fueron producidos por plantas invasoras fijadoras de N_2 (Malcolm et al. 2008; Elgersma et al. 2011; Von Holle et al. 2013; Grove et al. 2015).

Los impactos sobre las especies nativas y los ecosistemas invadidos pueden variar en función de la especie de planta exótica, de su edad y densidad (Lawrence et al. 1991; Gómez-Aparicio y Canham 2008a), así como de las propiedades abióticas y bióticas del hábitat o ecosistema invadido (Chytrý et al. 2008; Dassonville et al. 2008; Pyšek et al. 2008). Por ejemplo, aunque se ha generalizado que las plantas fijadoras de N_2 aumentan la concentración de N del suelo y aceleran sus flujos (ej. Stock et al. 1995; Rice et al. 2004; De Marco et al. 2013), el estudio de estas especies está sesgado a ecosistemas pobres en nutrientes, observándose efectos no tan claros en ecosistemas ricos en N como los bosques de ribera (Castro-Díez et al. 2009, 2012).

2.2. Mecanismos de impacto de las plantas exóticas invasoras

Los mecanismos por los cuales las especies exóticas invasoras (EEI) producen impactos ecológicos tienen que ver con las causas que les permiten invadir los ecosistemas (Ricciardi et al. 2013). Por tanto, las plantas invasoras producen impactos debido a que presentan características diferentes a las de

las especies nativas, abundan en el ecosistema o compiten mejor por los recursos, ya sea porque ocupan un nicho vacío en el ecosistema, porque tienen menor o ninguna presión de sus enemigos o porque presentan compuestos nuevos que afectan en gran medida a las especies nativas (Levine et al. 2003; Ricciardi et al 2013; Skurski et al. 2014). Estos mecanismos no son excluyentes y pueden actuar al mismo tiempo (Lamarque et al. 2011; Skurski et al. 2014).

A nivel de especie, las plantas invasoras pueden disminuir el crecimiento y reproducción de las plantas nativas mediante la interrupción de las interacciones planta-polinizador (menor número de visitas de polinizadores en plantas nativas). Esta interrupción tiene lugar por una producción de mayor cantidad o diferente tipo de néctar por parte de las plantas invasoras (Chittka y Schürkens 2001; Skurski et al. 2014). También, las plantas invasoras pueden reducir el crecimiento de plantas nativas interfiriendo en sus relaciones mutualistas con bacterias y hongos del suelo (bacterias fijadoras de N_2, micorrizas arbusculares y ectomicorrizas) (Reinhart y Callaway 2006; Stinson et al. 2006; Rodríguez-Echeverría et al. 2012). Por otro lado, una mejor competencia por recursos, como la luz, el agua o los nutrientes, permite a las plantas invasoras producir impactos en las comunidades de plantas nativas (Sala et al. 1996; Levine et al. 2003; Coleman y Levine 2007; Skurski et al. 2014).

Las plantas exóticas invasoras que difieren de las nativas en las estrategias de uso del N, así como en la composición química de sus tejidos o exudados de raíz, pueden afectar a la composición y actividad de microorganismos nativos (Wolfe y Klironomos 2005; Boudsocq et al. 2012; Lorenzo et al. 2013). Además, las plantas exóticas invasoras pueden afectar a plantas y microorganismos nativos a través de determinados compuestos químicos presentes en sus tejidos (Hierro y Callaway 2003). Los impactos en las propiedades del ecosistema pueden deberse a que las plantas invasoras presentan características nuevas, no presentes en el ecosistema receptor,

como la fijación de N_2, que implica cambios en los ciclos biogeoquimicos, sobretodo del N (Liao et al. 2008; Vilà et al. 2011; Castro-Díez et al. 2014b).

2.3. La hojarasca como mecanismo de impacto de las plantas exóticas invasoras

A través de la hojarasca, las plantas interactúan fuertemente con el medio abiótico y biótico, influyendo en los ciclos de nutrientes y en la estructura de las comunidades de plantas y organismos detritívoros (Facelli y Pickett 1991; Wallace et al. 1997; Xiong y Nilson 1999). Así, la caída de hojarasca (i. e. senescencia de diversos órganos de las plantas) es un proceso clave del ciclo de nutrientes, ya que permite que parte de los nutrientes utilizados por las plantas vuelvan al suelo (Vitousek 1984). De hecho, más del 90 % de la producción global de las plantas terrestres se convierte en MO muerta (Cebrián 1999). Además, la cantidad de hojarasca producida por diferentes especies vegetales se ha relacionado positivamente con las concentraciones de carbono y N en el suelo (Xu et al. 2013)

La capa de hojarasca del suelo puede afectar tanto positiva como negativamente a la germinación de especies de plantas. En primer lugar, la hojarasca que se acumula en el suelo supone una barrera física que intercepta la luz del sol y en general afecta negativamente a la germinación (Facelli y Picket 1991; Kostel-Hughes et al. 2005; Farrer y Goldberg 2009). Además, mediante lixiviación o descomposición, la hojarasca puede liberar compuestos químicos que pueden inhibir la germinación y crecimiento de determinadas especies de plantas (Jäderlund et al. 1996; Nasir et al. 2005; Valera-Burgos et al. 2012). Por otro lado, la hojarasca suaviza las temperaturas y preserva la humedad, características que favorecen la germinación de las semillas (Facelli y Picket 1991). No obstante, diferentes especies responden de forma distinta a la modificación del ambiente por la hojarasca, lo que convierte a la hojarasca en un agente de estructuración de

las comunidades de plantas (Facelli y Picket 1991; Xiong y Nilsson 1999; Koorem et al. 2011).

La calidad de la hojarasca se refiere a características físico-químicas tales como la dureza, el contenido en nutrientes (N y P), compuestos secundarios (ej. fenoles) o estructurales (ej. lignina) (Melillo et al. 1982; Ostrofsky 1997; Leroy et al. 2006). Así, las hojarascas blandas, con elevado contenido en nutrientes y bajo contenido en compuestos secundarios y estructurales, se consideran de mayor calidad, y por tanto son preferentemente consumidas por los organismos descomponedores (Quinn et al. 2000; Going y Dudley 2008). Esto ocasiona que sean descompuestas con mayor rapidez (Enriquez et al. 1993; Hladyz et al. 2009). Además, la colonización microbiana de la hojarasca (i. e. condicionamiento microbiano) determina un mayor consumo por parte de los macroinvertebrados descomponedores en ecosistemas acuáticos (Arsuffi y Suberkropp 1989; Wright y Covich 2005) y terrestres (Hanlon y Anderson 1979). La calidad de la hojarasca se ha considerado como el principal factor que determina la tasa de descomposición de la hojarasca tanto en los ecosistemas terrestres como en los acuáticos (Melillo et al. 1982; Abelho 2001). Por tanto, cambios en la calidad de la hojarasca pueden afectar a las comunidades de macroinvertebrados tanto terrestres (Bastow et al. 2008) como acuáticos (Sampaio et al. 2004), así como alterar las tasas de descomposición de la hojarasca (Hladyz et al. 2009).

El efecto de la hojarasca sobre el ciclo de nutrientes y las comunidades de plantas y detritívoros está influenciado por el momento temporal en que la hojarasca cae al suelo. No obstante, la cantidad y calidad de la hojarasca ha sido más estudiada que su dinámica de caída temporal, a pesar de la importancia que ésta tiene para el funcionamiento del ecosistema. El momento temporal en que cae la hojarasca determina las condiciones ambientales en las que será descompuesta en el suelo (Kirschbaum 2010) y la etapa del ciclo vital de plantas y otros organismos que se ve afectada. Por

ejemplo, el efecto de la hojarasca sobre las plantas será mayor si cae coincidiendo con etapas más vulnerables del ciclo de vida de las plantas, como son la germinación y el establecimiento (Facelli y Picket 1991). También, la dinámica temporal de caída de hojarasca determina el periodo de disponibilidad de alimento para los organismos descomponedores (Abelho y Graça 1996). El estudio de la dinámica temporal de caída de hojarasca se ha centrado principalmente en el componente mayoritario de la misma, que son las hojas senescentes (González-Muñoz et al. 2013). Sin embargo, la senescencia de otros órganos de las plantas puede tener también un importante papel en diversos componentes del ecosistema. Por ejemplo, las flores pueden presentar compuestos alelopáticos (Hosni et al. 2013) y suponen una importante entrada de nutrientes al suelo debido a su rápida descomposición y elevado contenido en nutrientes, como el P (Lee et al. 2011). El estudio aislado de uno de los componentes de la hojarasca ofrece una comprensión parcial del proceso de caída de hojarasca y de su impacto en el suelo.

La producción de hojarasca, el momento temporal en que cae, y sus características físico-químicas dependen en gran medida de las especies (Melillo et al. 1982; Bray y Gorham 1964). Por tanto, la invasión de ecosistemas por especies de plantas exóticas puede suponer cambios en la cantidad, calidad y dinámica de caída de la hojarasca, lo que a su vez puede tener efectos sobre los ciclos de nutrientes y las comunidades nativas de plantas y detritívoros. De hecho, la hojarasca se considera como uno de los mecanismos más importantes por el cual las plantas invasoras producen diversos impactos, como la modificación del microambiente para la germinación de semillas (Skurski et al. 2014).

2.4. Medida de los impactos de las plantas exóticas invasoras

Una de las formas más comunes de medir los impactos de las plantas invasoras en las comunidades nativas y en las propiedades del ecosistema es comparar los mismos parámetros en parcelas invadidas y no invadidas, que estén lo suficientemente juntas para asegurar que las condiciones microclimáticas son similares, y por tanto las diferencias observadas puedan atribuirse principalmente a las especies invasoras (Vilà et al. 2006; Hejda et al. 2009; Martin et al. 2009). Además, los estudios de invernadero en los que se miden diferentes variables en suelos en los que han crecido especies invasoras y nativas son también muy útiles para estudiar los impactos de las plantas invasoras en diferentes propiedades del suelo (Ehrenfeld et al. 2001; Kourtev et al. 2003). Del mismo modo, la comparación de los efectos que producen plantas invasoras y nativas en diferentes especies, comunidades y procesos del ecosistema permiten relativizar la intensidad del impacto de las especies invasoras (Catalán et al. 2013; Del Fabbro y Prati 2015; Shannon-Firestone y Firestone 2015). Sin embargo, existen numerosos estudios que establecen los efectos de la especie invasora de forma aislada, sin tener en cuenta los efectos que las especies nativas pueden tener también sobre las mismas especies o procesos (ej. Wardle et al. 1991; Bauer et al. 2012; Baratelli et al. 2012; Pal et al. 2015).

3. Ecosistemas fluviales y de ribera

Los ecosistemas fluviales presentan características abióticas y bióticas diferentes a las de otros ecosistemas acuáticos, lo que se debe principalmente a su elevado dinamismo originado por la presencia una corriente unidireccional (Allan y Castillo 2007). La dinámica de inundaciones de los ríos tiene una elevada importancia ecológica, ya que genera heterogeneidad espacial, la cual influye en las comunidades bióticas del río y en la vegetación de la ribera. Esto es especialmente importante en climas templados, donde la

estacionalidad produce periodos muy marcados de avenidas y sequías en los ecosistemas fluviales (Sabater y Elosegui 2009). La organización espacial heterogénea crea un mosaico de hábitats que favorece una elevada diversidad en la zona de ribera (Naiman y Decamps 1997).

El carácter unidireccional de los ríos implica también diferentes características físico-químicas y distintas comunidades bióticas en tramos altos, medios y bajos según la teoría del río continuo (Vannote et al. 1980; Sabater y Elosegui 2009). En los ríos de cabecera, la anchura del cauce es menor que en los tramos medios y bajos, con lo cual el dosel de la vegetación de ribera limita considerablemente la entrada de luz y, por tanto, la productividad primaria. Esto determina una menor abundancia de las comunidades de productores primarios y mayor abundancia de organismos heterótrofos en los ríos de cabecera (Sabater y Elosegui 2009). Asimismo, la menor abundancia de productores primarios en ríos de cabecera implica una mayor dependencia de los heterótrofos acuáticos por los aportes de materia orgánica (MO) del bosque de ribera (Wallace et al. 1997; Gessner et al. 1999).

Los macroinvertebrados (aquellos invertebrados retenidos por una malla de 500 µm) son los principales consumidores de hojarasca en el río, sobre todo los grupos funcionales denominados desmenuzadores o fragmentadores de la MO (Allan y Castillo 2007; Rodrigues-Capítulo et al. 2009). Además, la abrasión física producida por la corriente de agua y las comunidades microbianas (hongos y bacterias) tienen también un papel fundamental en la descomposición de la hojarasca en los ecosistemas fluviales (Webster y Benfield 1986). Los hongos colonizadores de hojarasca están especializados en la descomposición de compuestos como la lignina o la celulosa, mientras que las bacterias presentan enzimas que descomponen principalmente los polisacáridos (Pozo et al. 2009).

Los ecosistemas de ribera están asentados en las llanuras aluviales, extendiéndose desde el margen de las masas de agua continentales hasta el borde de los sistemas de ladera (Naiman y Decámps 1997). Estos ecosistemas

tienen un elevado valor ecológico, económico y social. Desde un punto de vista ecológico, los sotos o bosques de ribera atenúan los efectos de las crecidas y controlan los nutrientes, contaminantes y sedimentos que llegan al río (Naiman y Decámps 1997; Sabater et al. 2009). También, los bosques de ribera permiten la conectividad entre los ecosistemas terrestres y acuáticos, proporcionan refugio para las especies terrestres, constituyen corredores para el movimiento de fauna y suponen una importante fuente de alimento para organismos terrestres y acuáticos (Sterling 1996; Wallace et al. 1997; Decámps et al. 2009).

La población humana ha estado siempre muy ligada a las zonas de ribera, ya que de los ríos se obtiene agua para consumo o regadío, alimento o energía eléctrica (Sabater y Elosegui 2009). Al mismo tiempo, las zonas de ribera suponen lugares de ocio y esparcimiento para los ciudadanos (Castro Díez et al. 2001; Richardson et al. 2007). Sin embargo, la construcción de presas, canalización de los cauces de agua, o la contaminación por el uso de fertilizantes agrícolas y vertidos de aguas residuales ha supuesto la degradación de los ecosistemas fluviales y de ribera (Ward y Stanford 1995; Vitousek et al. 1997). La presencia de especies exóticas invasoras supone una amenaza más para estos ecosistemas (Richardson et al. 2007). Por ello, la presencia de plantas invasoras se considera un aspecto negativo en los índices de valoración de la calidad del bosque de ribera (Jáimez-Cuéllar et al. 2002). Las plantas que invaden ecosistemas de ribera producen impactos tanto en el ecosistema terrestre como acuático, afectando al proceso de reciclado de nutrientes y a las comunidades bióticas (Castro-Díez et al. 2009, 2012; Alonso et al. 2010). A pesar de estar profundamente interrelacionados, los ecosistemas acuáticos y terrestres se estudian habitualmente de forma separada en ecología (Stergiou et al. 2005).

3.1. Vulnerabilidad de los ecosistemas de ribera a la invasión por EEI

Determinadas características de los ecosistemas de ribera que favorecen una elevada diversidad de especies nativas también pueden favorecer la invasión por especies exóticas (EE) (Stohlgren et al. 1999; Levine et al. 2000). Por ejemplo, la disponibilidad de agua, así como unas temperaturas más suaves que en zonas circundantes, son características de los ecosistemas de ribera especialmente favorables en climas mediterráneos, caracterizados por altas temperaturas y escasa precipitación estival (Chytrý et al. 2008). La continua perturbación de los ecosistemas de ribera por el ser humano también favorece la invasión, en parte porque supone la destrucción de la vegetación, lo que deja espacios vacíos para el establecimiento de las EE (Lindig-Cisneros y Zedler 2001; Zedler y Kercher 2004; Richardson et al. 2007). Además, la cercanía de las poblaciones a las zonas de ribera ha tenido como consecuencia una elevada presión de propágulos, la cual ha favorecido la dispersión de algunas plantas introducidas en las ciudades con fines ornamentales (Kowarik y Säumel 2007; García-Berthou et al. 2008). Por todo ello, los ecosistemas de ribera se consideran especialmente vulnerables a la invasión de plantas exóticas (Richardson et al. 2007; Chitrý et al. 2008).

4. Dos árboles invasores de las riberas; *Ailanthus altissima* (Mill.) Swingle y *Robinia pseudoacacia* L.

Ailanthus altissima (Mill.) Swingle (Simaroubaceae) (árbol del cielo o ailanto) y *Robinia pseudoacacia* L. (Fabaceae) (falsa acacia) son dos árboles éxoticos invasores ampliamente distribuidos por zonas templadas del mundo (Kowarik y Säumel 2007; Cierjacks et al. 2013) (Figura 4). *A. altissima* es nativo de China mientras que *R. pseudoacacia* procede de Norte América, de las montañas Apalaches (Sanz-Elorza et al. 2004) (Figura 3).

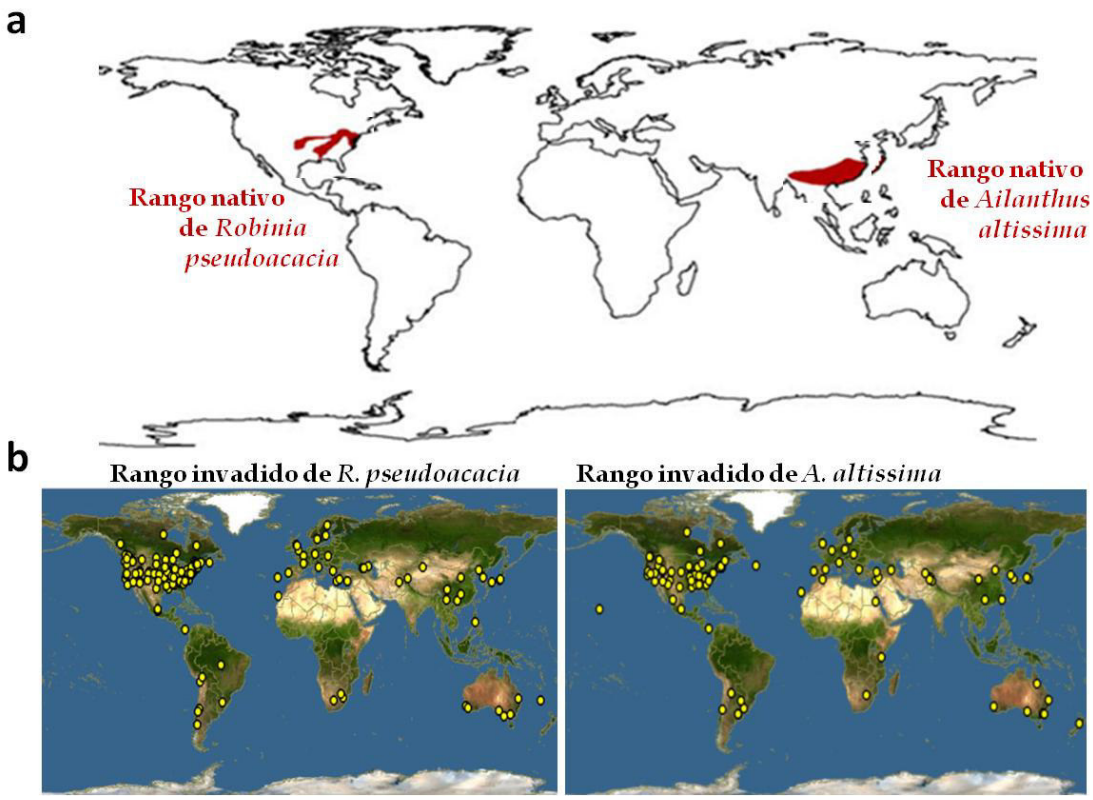


Figura 3. Rango nativo (a) e invadido (b) de las especies arbóreas *Ailanthus altissima* y *Robinia pseudoacacia*. (Modificado de Sanz-Elorza et al. 2004 (a) y http://www.discoverlife.org (b).

Fueron introducidos por primera vez en Francia, *R. pseudoacacia* sobre el año 1635 y *A. altissima* sobre 1740 (Sanz-Elorza et al. 2004). Posteriormente fueron plantados en otros países como árboles ornamentales en las ciudades o para otros usos como el control de erosión de taludes, los alineamientos de las carreteras, la producción de madera y miel, etc. (Kowarik y Säumel 2007; Cierjacks et al. 2013). Actualmente, tanto *A. altissima* como *R. pseudoacacia* crecen en un amplio rango de ambientes desde urbanos a naturales, apareciendo en bordes de carretera y vías de tren, en campos de cultivos y en ecosistemas de ribera (Sanz-Elorza et al. 2004; Figura 4). En la Península Ibérica co-ocurren con especies nativas propias de ribera, como *Fraxinus angustifolia*, *Populus alba* y *Ulmus minor* (González-Muñoz et al. 2014).

Figura 4. Arriba a la izquierda, un individuo adulto de *Ailanthus altissima*. Arriba a la derecha, individuos adultos de *Robinia pseudoacacia*. Abajo a la izquierda, detalle de las hojas de *A. altissima* en la ribera del Río Henares. Abajo a la derecha, detalle de las flores y hojas de *R. pseudoacacia*.

R. pseudoacacia es capaz de fijar N atmosférico en asociación mutualista con bacterias del género *Rhizobium* de forma muy eficiente (Moshki y Lamersdorf 2011). Esta capacidad le permite aumentar las concentraciones de N total y mineral del suelo así como incrementar las tasas de mineralización del N en suelos pobres en nutrientes (Rice et al. 2004). Estos efectos pueden facilitar la aparición de otras plantas exóticas y perdurar al menos 14 años tras la eliminación de *R. pseudoacacia* (Von Holle et al. 2006; 2013). Además, *R.*

pseudoacacia aumenta las tasas de descomposición de hojarasca respecto de especies de ecosistemas pobres en N (Lee et al. 2011), mientras que las disminuye respecto de especies de ecosistemas de ribera (Castro-Díez 2009; Alonso et al. 2010; Castro-Díez et al. 2012). Por su lado, *A. altissima* puede afectar al crecimiento de diferentes especies de árboles nativos a través de la alelopatía (Gómez-Aparicio y Canham 2008a), así como disminuir la diversidad de plantas (Motard et al. 2011), alterar las comunidades de artrópodos del suelo (Gutiérrez-López et al. 2014; Motard et al. 2015), aumentar el N del suelo (Vilà et al. 2006) o acelerar la descomposición de hojarasca en diferentes ecosistemas (Swan et al. 2008; Castro-Díez et al. 2009; Alonso et al. 2010).

Debido a su elevada capacidad de dispersión y colonización de zonas naturales, así como a los impactos que producen y a la dificultad para erradicarlas, *A. altissima* y *R. pseudoacacia* están consideradas dentro de las 100 peores EEI en Europa (DAISIE 2009) y dentro de las 20 más dañinas de España (GEIB 2006). Sin embargo, existen importantes vacíos de conocimiento en cuanto a los impactos que estas especies pueden producir en los ecosistemas y comunidades de ribera (Tabla 1 y 2). Por ejemplo, se desconoce su efecto en el proceso global de caída de hojarasca (cantidad, calidad y dinámica de caída de diferentes órganos senescentes), que es un proceso clave para el reciclado de nutrientes y la estructura de las comunidades vegetales, animales y microbianas. Aunque se ha estudiado su efecto en las tasas de descomposición de ecosistemas terrestres y acuáticos lénticos, su efecto en ecosistemas fluviales es escaso (pero ver Swan et al. 2008). Tampoco se ha establecido el efecto que pueden tener estos árboles invasores en las comunidades de los ríos de cabecera, las cuales dependen fuertemente de los aportes hojarasca de la vegetación de ribera. Además, se desconoce en gran medida el impacto que *A. altissima* y *R. pseudoacacia* pueden causar sobre las comunidades microbianas acuáticas y terrestres (bacterias y hongos), cuyo papel es fundamental en los ciclos de nutrientes y las tasas de descomposición de hojarasca. Por otro lado, los efectos

alelopáticos que pueden tener los lixiviados de hojarasca de estas especies invasoras sobre las plantas nativas de ribera no han sido establecidos en los suelos de ribera. El conocimiento integral de los impactos de *A. altissima* y *R. pseudoacacia* en los ecosistemas de ribera es de gran importancia para la toma de decisiones en cuanto a la gestión de estas especies invasoras, así como para la evaluación de riesgos en zonas donde son potencialmente invasoras (Cabra-Rivas et al. 2016). Esta Tesis aborda el estudio de los impactos de *A altissima* y *R pseudoacacia* en los ecosistemas de ribera y fluviales, que como ya se ha mencionado, constituye un cuestión relevante desde un punto de vista socio-ecológico, y es a su vez un tema relativamente desconocido.

Tabla 1. Principales impactos estudiados en la especie *Ailanthus altissima* a diferentes niveles (Ecosistema, comunidad y especie). Los cuadros en azul resaltan los vacíos de conocimiento, algunos de los cuales se abordan en esta tesis. El impacto se refiere a la comparación con el estado pre-invasión o con las especies nativas, según el caso. Para cada variable el valor en la especie invasora puede ser mayor (>), menor (<), igual (=) o diferente cuando no aplica el sentido de la diferencia (≠). PME: fosfomonoesterasa, N: nitrógeno, P: fósforo, Ca: Calcio, Mg: magnesio, K: potasio, MO: materia orgánica.

Nivel de organización		Componente o proceso del ecosistema	Variable medida	Impacto	Referencias
	Ecosistema	Caída de la hojarasca	Cantidad de hojarasca	>	González-Muñoz et al. 2013
			Dinámica temporal de caída de diferentes órganos senescentes		
			Calidad de la hojarasca	=	Castro-Díez et al. 2009; Alonso et al. 2010; Castro-Díez et al. 2012
		Descomposición de hojarasca	Tasa en medio acuático	<	Alonso et al. 2010
				>	Swan et al. 2008
			Tasa en medio terrestre	>	Castro-Díez et al. 2009; 2012
		Nutrientes del suelo	Concentración de nutrientes disponibles (N, Ca, Mg, K)	>	Gómez-Aparicio y Canham 2008;
			Concentración de N total	>	Vilà et al. 2006
			Concentración de N y MO	=	Castro-Díez et al. 2009; 2012
		Mineralización de N en el suelo	Tasas netas de nitrificación y mineralización del N	>	Gomez-Aparicio y Canham 2008
			Tasas netas de nitrificación y mineralización del N	=	Castro-Díez et al. 2009
		Mineralización de P en el suelo	Actividad PME		
		Mineralización del Ca en el suelo	Tasa de mineralización del Ca	>	Gómez-Aparicio y Canham 2008
		Iones del suelo	pH	>	Vilà et al. 2006; Gomez-Aparicio y Canham 2008
				=	Castro-Díez et al. 2009; 2012
	Comunidad	Vegetal	Diversidad y riqueza de plantas nativas	<	Vilà et al. 2006; Motard et al. 2011; Constán-Nava et al. 2015
				>	Fotiadis et al. 2011
		Microbiana			
		Animal	Composición específica de artrópodos del suelo	≠	Gutiérrez-López et al. 2014; Motard et al. 2015
			Composición de familias de macroinvertebrados acuáticos	=	Alonso et al. 2010
			Actividad descomponedora de los macroinvertebrados fragmentadores	>	Swan et al. 2008
	Especie	Vegetal	Germinación y crecimiento radicular de plantas nativas en laboratorio	<	Lawrence et al. 1991; De Feo et al. 2003; Catalán et al. 2013
			Crecimiento de las especies de árboles nativos en campo	<	Gómez-Aparicio y Canham 2008
		Microbiana	Crecimiento de *Escherichia coli*, *Staphylococcus aureus y Pseudomonas aeruginosa* (En este caso no hay comparación con especies nativas)	<	Huo et al. 2012
		Animal			

Tabla 2. Principales impactos estudiados en la especie *Robinia pseudoacacia* a diferentes niveles (ecosistema, comunidad y especie). Los cuadros en azul resaltan las lagunas de conocimiento, algunas de los cuales se abordan en esta tesis. El impacto se refiere a la comparación con el estado pre-invasión o con las especies nativas, según el caso. Para cada variable el valor en la especie invasora puede ser mayor (>), menor (<), igual (=) o diferente cuando no aplica el sentido de la diferencia (≠). PME: fosfomonoesterasa, N: nitrógeno: Ca: Calcio, Mg: magnesio, K: potasio, MO: materia orgánica.

Nivel de organización		Componente o proceso del ecosistema	Variable medida	Impacto	Referencias
	Ecosistema	Caída de la hojarasca	Cantidad de hojarasca	< >	González-Muñoz et al. 2013 Rice et al. 2004
			Dinámica temporal de caída de diferentes órganos senescentes	=	Lee et al. 2010
			Calidad de la hojarasca - Porcentaje de lignina y el ratio lignina:N - C:N	 > <	 Castro-Díez et al. 2009; Alonso et al. 2010; Castro-Díez et al. 2012
		Descomposición de hojarasca	Tasa en medio acuático	<	Alonso et al. 2010
			Tasa en medio terrestre	> <	Lee et al. 2010; Buzhdigan et al. 2016 Castro-Díez et al. 2009; 2012
		Nutrientes del suelo	Concentración de N, P, Ca, Mg, MO, nitrato, amonio	>	Rice et al. 2004; Malcolm et al. 2008; Von Holle et al. 2013; Staska et al. 2014;
			Concentración de N y MO	=	Castro-Díez et al. 2009; 2012
		Mineralización del N en el suelo	Tasas netas de nitrificación y de mineralización del N	> =	Rice et al. 2004; Malcolm et al. 2008; Von Holle et al. 2013; Buzhdigan et al. 2015 Castro-Díez et al. 2009; 2012
		Mineralización del P en el suelo	Actividad PME		
		Iones del suelo	pH	=	Castro-Díez et al. 2009; 2012
	Comunidad	Vegetal	Riqueza especies exóticas	>	Von Holle et al. 2006
			Abundancia de especies nitrófilas	>	Staska et al. 2014
		Microbiana	Composición de bacterias del suelo	=	Taniguchi et al. 2009
			Riqueza y diversidad de bacterias y hongos micorrícicos arbusculares	> <	Feng et al. 2011 Taniguchi et al. 2007
			Riqueza, diversidad y abundancia de hongos ectomicorrícicos		
		Animal	Composición de familias de macroinvertebrados acuáticos	=	Alonso et al. 2010
	Especie	Vegetal	Germinación y crecimiento radicular de plantas nativas en laboratorio	<	Nasir et al. 2005; Catalán et al. 2013
		Microbiana			
		Animal			

5. Objetivos, hipótesis y estructura de la Tesis

5.1. Objetivo e hipótesis general

El objetivo general de esta Tesis Doctoral es evaluar los impactos ecológicos de los árboles exóticos invasores, *Ailanthus altissima* y *Robinia pseudoacacia,* sobre propiedades bióticas y abióticas de los ecosistemas fluviales y riparios desde un enfoque integrado. Se evaluarán los impactos sobre tres niveles de organización: ecosistema, comunidad y especie. Se espera que los árboles exóticos, *A. altissima* y *R. pseudoacacia,* a través de la producción de hojarasca provoquen cambios en los ecosistemas de ribera que invaden, tanto en medio acuático como terrestre, y en todos los niveles de organización estudiados.

5.2. Objetivos específicos

Los objetivos particulares que se abordan son los siguientes:

- Evaluar el efecto de los árboles invasores, *A. altissima* y *R. pseudoacacia,* en la cantidad, calidad y momento de caída de la hojarasca en ecosistemas de ribera del Centro Peninsular (*Capítulo 2*).
- Establecer si existen diferencias en las propiedades del suelo, concentración de materia orgánica (MO), nitrógeno (N), fósforo (P) y pH de ecosistemas de ribera nativos e invadidos por los árboles exóticos, *A. altissima* y *R. pseudoacacia* (*Capítulo 2*).
- Valorar el impacto de los árboles invasores, *A. altissima* y *R. pseudoacacia,* en la riqueza y diversidad de bacterias del suelo, la MO, el P total, el N total y mineral, así como en las tasas de mineralización de N y P del suelo, siguiendo dos aproximaciones: muestreo de suelo en campo en parcelas pareadas invadida-nativa y crecimiento de las especies nativas e invasoras en invernadero (*Capítulo 3*).
- Evaluar el efecto de extractos acuosos de hojas senescentes de las especies invasoras (*A. altissima* y *R. pseudoacacia*) y nativas (*Fraxinus*

angustifolia y *Populus alba*) sobre la germinación y crecimiento radicular de varias especies del sotobosque en suelos de ribera (*Capítulo 4*).

- Evaluar el potencial efecto de la hojarasca de *A. altissima* y *R. pseudoacacia* sobre las tasas de descomposición y las comunidades de hongos y macroinvertebrados de un ecosistema fluvial (*Capítulo 5*).

5.3. Hipótesis específicas

Las hipótesis que se testan son las siguientes:

- Las elevadas tasas de crecimiento de los árboles invasores, *A. altissima* y *R. pseudoacacia,* determinarán la producción de mayores cantidades de hojarasca, y por tanto mayor cantidad de MO y nutrientes en el suelo (*Capítulo 2*).
- *R. pseudoacacia* producirá una hojarasca con mayor concentración de N debido a su capacidad de fijación del N atmosférico en asociación mutualista con bacterias. Del mismo modo, es esperable una mayor concentración de N total y mineral en suelos invadidos por *R. pseudoacacia* debido al aporte de hojarasca más rica en N y al aporte directo procedente de la fijación del N atmosférico (*Capítulo 2 y 3*).
- A nivel de comunidad y especie, la invasión de los ecosistemas de ribera por los árboles invasores, *A. altissima* y *R. pseudoacacia,* implica la entrada de hojarasca con diferentes propiedades físico-químicas respecto de la hojarasca de especies nativas en el suelo y en el río. Según la *Hipótesis de las Nuevas Armas* (Callaway y Ridenour 2004), las especies de bacterias del suelo, de plantas del sotobosque, así como las especies de macroinvertebrados y de hongos del río, no han co-evolucionado con las especies de árboles exóticos invasores, por lo que se verán afectadas por los nuevos compuestos presentes en su hojarasca (*Capítulos 3, 4 y 5*). Esto se verá reflejado en:

- Una comunidad de bacterias del suelo diferente en sitios invadidos y no invadidos por las especies exóticas. Los "nuevos" compuestos presentes en las especies invasoras afectarán a los microorganismos del suelo relacionados con los ciclos de nutrientes (ej. bacteria nitrificantes, desnitrificantes o amonificantes), y por tanto afectarán a las tasas de mineralización de nutrientes del suelo (*Capítulo 3*).
- Un mayor potencial alelopático de la hojarasca exótica respecto de la nativa sobre diferentes especies de plantas del sotobosque (*Capítulo 4*).
- Una menor colonización fúngica y una estructura de la comunidad de macroinvertebrados diferente en la hojarasca exótica respecto de la nativa, lo que a su vez determinará menores tasas de descomposición de la hojarasca exótica en el ecosistema fluvial. Sin embargo, también se contempla la posibilidad de que las propiedades físico-químicas de cada hojarasca determinen mejor sus tasas de descomposición que su origen exótico o nativo (*Capítulo 5*).

5.4. Estructura de la Tesis

Esta Tesis Doctoral se ha estructurado en 8 capítulos. Al presente capítulo de introducción general le siguen 4 capítulos de investigación, los cuales tratan de responder a los objetivos específicos mencionados anteriormente. Estos capítulos se corresponden con artículos científicos publicados (*Capítulos 2, 3 y 5)* o enviados (*Capítulo 4*) a revistas científicas indexadas (pertenecientes a la base de datos Science Citation Index (SCI)). Los capítulos de investigación están escritos en inglés con sus correspondientes apartados de Resumen, Introducción, Material y Métodos, Resultados, Discusión y Bibliografía.

En el **Capítulo 2**, "*Do the invasive trees,* Ailanthus altissima *and* Robinia pseudoacacia*, alter litterfall dynamics and soil properties of riparian ecosystems in Central Spain?"*, se trata de establecer el efecto de los árboles invasores *A. altissima* y *R. pseudoacacia* en el proceso de caída de hojarasca (Figura 4) y en

las propiedades del suelo de ribera. Para ello se compara durante 2 años la cantidad, calidad y el momento de caída de hojarasca de bosques de ribera invadidos por *A. altissima* y *R. pseudoacacia* y bosques de ribera nativos, dominados por las especies, *Fraxinus angustifolia* y *Populus alba*. Se tienen en cuenta diferentes órganos senescentes (hojas, flores, frutos, ramas). Además se compara el contenido en materia orgánica (MO) y nutrientes (N y P) de cada bosque. Se discute la posible influencia del proceso de caída de hojarasca en las propiedades del suelo (MO, N y P). También se discuten las consecuencias de los cambios en el proceso de caída de hojarasca para las comunidades de plantas y detritívoros.

En el **Capítulo 3**, "*Impacts of the alien trees* Ailanthus altissima *(Mill.) Swingle and* Robinia pseudoacacia *L. on soil nutrients and microbial communities*" se establece el impacto de los árboles invasores en determinadas propiedades del suelo (nutrientes, MO) y procesos del ciclo de nutrientes (tasas de mineralización neta del N y actividad fosfatasa), así como en la estructura de la comunidad de bacterias edáficas (Figura 4). Para ello, se comparan estos parámetros en suelos recogidos en el campo en parcelas pareadas invadida-nativa (*A. altissima*-*P. alba*; *R. pseudoacacia*-*P. alba*). Además se realiza una aproximación experimental en invernadero, creciendo las especies invasoras y la nativa, *P. alba,* en un mismo suelo durante 6 meses y midiendo después los mismos parámetros que en los suelos del campo. Esto permite asegurar si las diferencias encontradas en el campo se deben al crecimiento de las especies y no a características pre-existentes en cada sitio.

En el **Capítulo 4,** "*Allelopathic effects of exotic invasive and native trees on co-existing undercanopy species. The soil as modulator*", se establecen los efectos alelopáticos de extractos acuosos de hojas de árboles exóticos (*A. altissima* y *R. pseudoacacia*) y nativos (*P. alba* y *F. angustifolia*) sobre la germinación y crecimiento de radícula de diferentes especies de plantas del sotobosque (Figura 4), las cuales presentan diferentes formas de vida (hemicriptófitos, terófitos y fanerófitos), tanto herbáceas (anuales y perennes) como leñosas.

Los extractos acuosos tratan de simular los lixiviados procedentes de la hojarasca en periodos de precipitación. Los experimentos se realizan usando papel y suelo como substrato de germinación de semillas para establecer el efecto potencial y real de los extractos.

En el **Capítulo 5**, "*Decomposition and biological colonization of native and exotic leaf litter in a Central Spain stream*" se compara la descomposición de hojarasca de árboles exóticos (*A. altissima* y *R. pseudoacacia*) y nativos (*P. alba* y *F. angustifolia*) en un río de cabecera (Figura 4). Además se compara la colonización fúngica y por macroinvertebrados de las hojas exóticas y nativas (Figura 4).

En el **Capítulo 6** se resumen los resultados obtenidos en esta Tesis Doctoral y en el **Capítulo 7** se realiza una discusión general de los 4 capítulos de investigación (*Capítulos 2-5*). Por último, en el **Capítulo 8** se presentan las principales conclusiones de esta Tesis Doctoral.

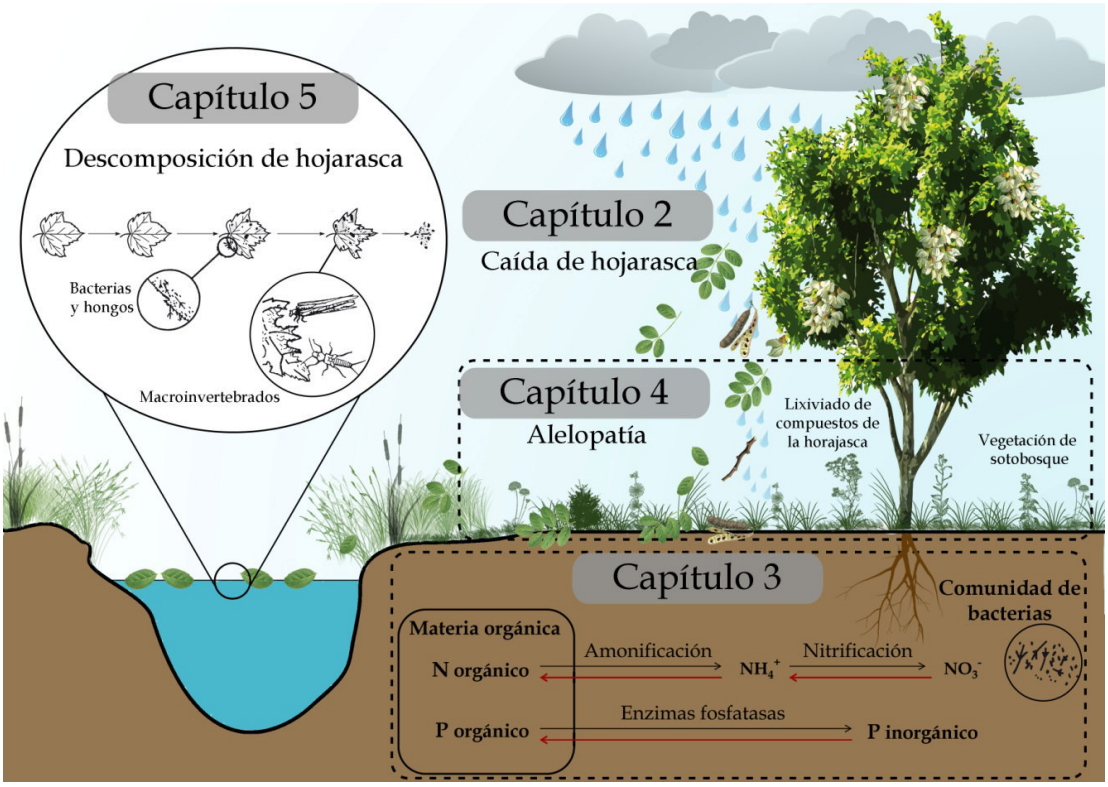


Figura 4. Esquema conceptual de la estructura de la Tesis Doctoral. Cada uno de los capítulos de la Tesis trata de abordar los impactos que la presencia de los árboles exóticos invasores (*Ailanthus altissima* y *Robinia pseudoacacia*) pueden producir en diferentes componentes y procesos de un ecosistema fluvial y de ribera. En el **Capítulo 2** se aborda el impacto en el proceso global de caída de hojarasca. En el **Capítulo 3** se cuantifica el impacto en las tasas de mineralización del N y del P, así como en la comunidad de bacterias edáficas. En el **Capítulo 4** se establece el efecto alelopático que pueden tener los compuestos lixiviados de la hojarasca, por acción de la lluvia, sobre las especies de plantas del sotobosque. En el **Capítulo 5** se estudia el efecto de la entrada de hojarasca en el río, específicamente en las tasas de descomposición y en las comunidades de hongos y macroinvertebrados acuáticos.

Capítulo 2

Do the invasive trees, *Ailanthus altissima* and *Robinia pseudoacacia*, alter litterfall dynamics and soil properties of riparian ecosystems in Central Spain?

El bosque sería muy triste si sólo cantaran los pájaros que mejor lo hacen.

Rabindranath Tagore

Este capítulo reproduce el texto del siguiente manuscrito:

This chapter reproduces the text of the following manuscript:

Medina-Villar S, Castro-Díez P, Alonso A, Cabra-Rivas I, Parker IM, Pérez-Corona E (2015) Do the invasive trees, *Ailanthus altissima* and *Robinia pseudoacacia*, alter litterfall dynamics and soil properties of riparian ecosystems in Central Spain?. Plant and Soil 396 (1): 311-324. doi: 10.1007/s11104-015-2592-4

Fotografía: Bosque de ribera invadido por la especie exótica, *Ailanthus altissima,* cerca de Chiloeches (Guadalajara, España). Puede verse una de las trampas de caída vertical utilizadas en este estudio para recoger la hojarasca.

Por: Silvia Medina Villar

Photography: Riparian forest invaded by *Ailanthus altissima* near Chiloeches (Guadalajara, Spain). The photo shows one of the vertical litter traps used in this study to collect the litter.

By: Silvia Medina Villar

Resumen

Alteran los árboles invasores, *Ailanthus altissima* y *Robinia pseudoacacia*, las dinámicas de caída de hojarasca y las propiedades del suelo de ecosistemas de ribera del centro de España?

Nuestro objetivo fué establecer el impacto de *Ailanthus altissima* y *Robinia pseudoacacia* en el proceso completo de caída de hojarasca y en las propiedades del suelo de ecosistemas riparios del centro de España comparando entre bosques invadidos y no invadidos. Seleccionamos 3-4 parcelas de cuatro tipos diferentes de bosques: invadidos por *A. altissima* o *R. pseudoacacia*, o dominados por los nativos *Fraxinus angustifolia* o *Populus alba*. En cada parcela recolectamos mensualmente durante dos años la hojarasca mediante trampas de caída vertical. Después separamos dicha hojarasca por especie y parte de la planta (hojas, flores, frutos y ramas). Después, calculamos las entradas anuales de hojarasca, nitrógeno (N) y fósforo (P) al suelo. También analizamos pH, la materia orgánica (MO), el N y el P del suelo. La mayoría de las fracciones de hojarasca procedentes de los árboles invasores contenían alrededor del doble de N que las procedentes del árbol nativo *P. alba*. Aunque las diferencias no fueron significativas, las entradas anuales de N en bosques invadidos fueron 1.7-2.2 veces mayores que en bosques nativos. En los bosques invadidos se observaron picos extra de caída de hojarasca en verano y/o primavera tardía (junio), que correspondieron con fracciones reproductivas y/u hojas. El porcentaje de hojarasca anual representado por estos picos fue alrededor del doble que los mismos en los bosques nativos. La cantidad de MO, y las concentraciones de N y de P del suelo fueron mayores en bosques invadidos que en bosques de *P. alba*. Además, los bosques invadidos por *R. pseudoacacia* presentaban una mayor cantidad de MO y concentración de N en el suelo que los bosques de *F. angustifolia*. Este estudio mostró la capacidad de *A. altissima* y *R. pseudoacacia* para alterar las propiedades del suelo y las dinámicas de caída de hojarasca en ecosistemas riparios.

Palabras clave: árboles exóticos; fósforo; fracciones de hojarasca; materia orgánica del suelo; patrones temporales de caída de hojarasca.

Abstract

Do the invasive trees, *Ailanthus altissima* and *Robinia pseudoacacia*, alter litterfall dynamics and soil properties of riparian ecosystems in Central Spain?

We aimed to assess the impacts of *A. altissima* and *R. pseudoacacia* on the whole litterfall process and on soil properties of riparian ecosystems of Central Spain by comparing invaded and non-invaded forests. We selected 3-4 plots of four different types of forests: invaded by *Ailanthus altissima* or *Robinia pseudoacacia*, or dominated by the native *Fraxinus angustifolia* or *Populus alba*. In each plot we collected the litter monthly over two years and separated the litter from different plant organs. We calculated annual inputs of litter, nitrogen (N) and phosphorus (P) to soil. We also analyzed soil pH, organic matter (OM), N and P. Most litter fractions from the two invasive trees contained over twice as much N as those from the native *P. alba*. Although not significantly different, the annual inputs of N in invaded forests were 1.7-2.2 times higher than in native forests. In the invaded forests, extra litterfall peaks were found in summer and/or late-spring (June), corresponding to reproductive and/or leaf litter fractions. The percentage of annual litter represented by these peaks was over twice those in native forests. Soil OM, N and P were higher in invaded than in *P. alba* forests. In addition, forests invaded by *R. pseudoacacia* had higher soil OM and N than *F. angustifolia* forests. This study showed the capability of *A. altissima* and *R. pseudoacacia* to alter soil properties and litterfall dynamics in riparian ecosystems.

Key-words: Exotic trees; litterfall timing; litter fractions; nitrogen; phosphorus; soil organic matter.

Introduction

Litterfall is a key process for nutrient cycling and energy transfer from plants to soils in terrestrial ecosystems (Vitousek 1984). The quality and quantity of plant litter play an important role in structuring the communities of plants and detritivorous invertebrates living in the soil (Facelli and Picket 1991; Ponge et al. 1993; Xiong and Nilsson 1999), and the aquatic food webs when the litter falls into aquatic ecosystems (Wallace et al. 1997). In addition, litterfall dynamics, i.e. the timing of abscission of different plant organs (e.g. leaves, flowers, branches), determines the period of food availability for detritivores, as well as the microclimatic conditions for litter decomposition (Abelho and Graca 1996; Singh et al. 1999; Kirschbaum 2010). The chemical composition of the litter, particularly nutrient content, largely determines the rate of litter decomposition (Simons and Seastedt 1999; Wang et al. 2008), as well as soil properties, such as organic matter (OM), nutrient concentrations and pH (Witkowski 1991; Rice et al. 2004; Yelenik et al. 2004). Given that litter production, litterfall dynamics and chemical composition of plant tissues vary across species (Bray and Gorham 1964; Facelli and Pickett 1991; Abelho 2001; Martin et al. 2009; González 2012), a change in the species composition of the plant community, e.g. by an exotic plant invasion, may alter the ecosystem processes and soil properties affected by litter properties.

Riparian ecosystems are highly vulnerable to exotic plant invasion in part due to their high frequency of disturbance as well as habitat heterogeneity, which provides more opportunities for exotic plants to find appropriate conditions to establish and persist (Hobbs and Huenneke 1992; Hood and Naiman 2000; Chytrý et al. 2008). The invasion by exotic plants implies changes in plant community composition, which in turn may affect the quantity, quality, and dynamics of litterfall and therefore the functioning of both aquatic and terrestrial ecosystems (Bray and Gorham 1964; Facelli and Pickett 1991; Abelho 2001). The magnitude and direction of the effect of exotic invasive trees on litterfall may depend on the invasive species and the invaded community

(Witkowski 1991; Ellis et al. 1998; Rice et al. 2004; Martin et al. 2009; Lee et al. 2011). Litter production in riparian forests can depend on soil properties, community structure and distance to the river (González et al. 2010). However, the link between invasion and soil properties through alteration of litterfall patterns remains largely unexplored.

Ailanthus altissima (Mill.) Swingle. (Simaroubaceae) and *Robinia pseudoacacia* L. (Fabaceae) are two exotic invasive trees natives to China and North America, respectively (Sanz-Elorza et al. 2004). They are found invading temperate ecosystems worldwide and are considered among the 100 worst invasive species in Europe (Kowarik and Säumel 2007; DAISIE 2009; Cierjacks et al. 2013). In Spain they were introduced during the 18th century for ornamental purposes, and they are now spreading through riparian areas, among other habitats, being considered among the 20 most harmful species in Spain (Sanz-Elorza et al. 2004; GEIB 2006). Both invaders are deciduous and in Central Spain they coexist with other riparian deciduous trees such as *Populus alba* L. (Salicaceae), *Populus nigra* L. (Salicaceae), or *Fraxinus angustifolia* Vahl. (Oleaceae) (De la Cruz et al. 1996; Castro-Díez et al. 2009). *R. pseudoacacia* is a N_2-fixing species capable of increasing N pools and speeding up N cycling in nutrient-poor invaded ecosystems (Rice et al. 2004; Von Holle et al. 2013). *A. altissima* can also increase soil N and pH in a range of different soils (Vilá et al. 2006; Gómez-Aparicio and Canham 2008b). In contrast, *Robinia pseudoacacia* is able to decrease soil pH due to enhanced nitrification (Vítková et al. 2015). In riparian ecosystems of Central Spain, *A. altissima* produced higher quantities of fast-decomposing leaf litter than coexisting native trees, while *R. pseudoacacia* had the contrary effect (Castro-Díez et al. 2009, 2012; González-Muñoz et al. 2013). Phenological studies indicated that both invasive species displayed later flowering and fruit set and earlier leaf abscission than most of the coexisting native trees in Central Spain (Castro-Díez et al. 2014c). Because other plant organs can be important sources of nutrients to soil (e.g. Lee et al. 2011), studies of litterfall that exclusively consider the leaf fraction provide only a partial understanding of the impact of plant invaders on soil inputs. Moreover,

seasonal variation of litterfall may be essential, but it is rarely studied because of the frequent and sustained sampling required. No one has yet estimated the effects of *A. altissima* and *R. pseudoacacia* on soil properties through the whole litterfall process, taking into account all plant organs, their nutrient content and the time when they fall.

The aim of this study was to assess the impacts of *A. altissima* and *R. pseudoacacia* on the whole litterfall process and on soil properties of riparian ecosystems of Central Spain by comparing invaded and non-invaded forests. Specifically we tested the effects of the invasive species on: 1) the annual quantity of litterfall from different plant organs and their temporal dynamics 2) nitrogen (N) and phosphorus (P) inputs to the soil from litterfall and their temporal dynamics 3) soil N and P concentration, OM and pH. We expected litter production in invaded forests to be greater than in non-invaded forests, given the higher growth rates and lower N use efficiency reported for these invaders as compared to the coexisting native trees (Kowarik and Säumel 2007; Cierjacks et al. 2013; González-Muñoz et al. 2013; Castro-Díez et al. 2014c). Associated with increased litter production, we expected higher inputs of nutrients and higher levels of soil nutrients and OM in invaded forests. We also expected different litterfall dynamics between invaded and native forests due to the contrasting phenologies of these exotic and native trees (Castro-Díez et al. 2014c). This study provides valuable data to understand the impacts produced by *A. altissima* and *R. pseudoacacia* in riparian Mediterranean forests on the whole litterfall process and thus on soil properties and the detritivore communities that rely on litter.

Materials and Methods

Study plots

The study was carried out within the riparian forest (along 80-km stretch) of the Henares River (Tagus Basin, Central Spain). In this area, the climate is continental Mediterranean, with a mean annual precipitation of 341 mm and mean annual temperature of 13 ºC during the two study years, and an arid period of at least two months (Fig. 1).

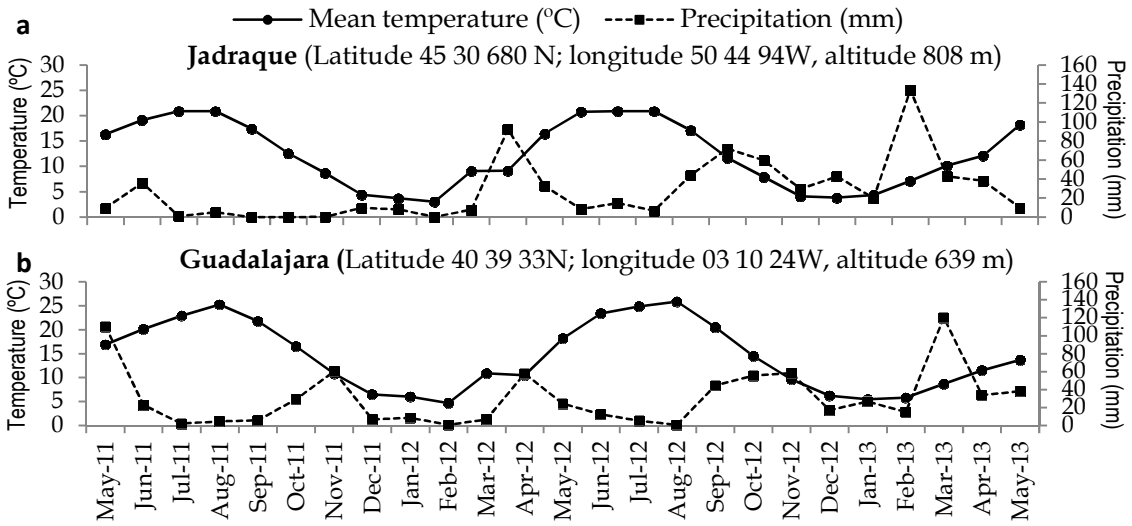


Figure 1. Mean monthly temperature and precipitation from the meteorological stations of Jadraque (a) and Guadalajara (b) during the study period (May 2011-May 2013). Data from the Ministry of *Agricultura Alimentación y Medio Ambiente* of Spain (http://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos and http://eportal.magrama.gob.es/websiar/SeleccionParametrosMap.aspx?dst=1)

Native vegetation is dominated by *P. alba*, *P. nigra*, *F. angustifolia*, *Salix alba* and *Ulmus minor*. Some areas are invaded by *A. altissima* and *R. pseudoacacia*, which may grow in pure stands or mixed with native species. We selected four forest types, i.e., patches of forests dominated by four different tree species: *A. altissima, R. pseudoacacia*, *F. angustifolia* and *P. alba* (**Anexo, foto 1**), occurring under similar environmental conditions (Xerothent + Xerofluvent soils; Fernández et al. 2012, 2013). For each forest type, we delimited three or four 300 m² plots where the target species was dominant (see Table 1). Plots were all

found within 100 m of the Henares River and within 80 km of each other. Plots had rectangular shape, with the wider side parallel to the river, except one *A. altissima* plot (AA1) which had triangular shape. In spring 2012 we measured in each plot the diameter at breast height (DBH) of all adult trees (DBH > 7 cm and height > 1.30 m) and calculated the total tree basal area (TTBA) ($\Sigma\Pi\left(\frac{DBH}{2}\right)^2$) and the percentage of TTBA belonging to the dominant species of each plot (DomTBA). In all plots, DomTBA represented more than 50 % of TTBA (Table 1). Average diameter at breast height (DBH) was similar among forest types (Table 1).

Litterfall collection

Eighteen litterfall traps were distributed within each plot. To collect different plant materials, we used three types of traps: 1) Four traps made of mosquito net (1 mm mesh) forming a square (0.30 m^2 surface) and fixed to the ground with spikes; 2) ten "pot" traps consisting of poly-vinyl chloride (PVC) circular pots (0.02 m^2 surface) fixed on the ground with spikes and containing a nylon bag (< 1 mm mesh); 3) four hanging traps consisting of nylon bags (< 1 mm mesh) fixed to a wire circle of 0.18 m^2 at 1 m above the ground (**Anexo, foto 2**). Overall, traps covered a total collection surface of 2.12 m^2 per plot and were evenly distributed within each plot.

We collected the contents of each trap monthly from 15th June 2011 to 15th May 2013. Once in the lab, the content of each sample were separated by tree species and plant litter fraction (leaves, bark and branches, flowers, fruits and unidentifiable debris). Then, samples were oven-dried at 60 ºC for at least 48 h and weighed (Balance Sartorius BP211D, Germany; 0.0001 g). We pooled all the data across the different traps for each plot to calculate monthly (g m^{-2} $month^{-1}$) and annual (g m^{-2} $year^{-1}$) litterfall for each fraction and for all litter.

Table 1. Summary of the community structure of adult trees in the study plots. For each plot, total number of adult trees (height ≥ 1.30 m and diameter at breast height (DBH) ≥ 7 cm) from dominant and non-dominant species (in brackets), total tree basal area (TTBA), mean (± SE) DBH of all adult trees, and the percentage of TTBA represented by the basal area of the dominant tree species (DomTBA) are presented.

Plot name	Location	Dominant tree species (number of adults)	Other tree species (number of adults)	TTBA (m^2)	Mean DBH (Mean (cm)	DomTBA (% from TTBA)
AA1	40º 34' 31.1'' N 3º 13' 46.6'' W	*Ailanthus altissima* (39)		7.1	14.2 ± 0.9	100.0
AA2	40º 34' 27.4'' N 3º 13' 46.4'' W	*A. altissima* (31)	*Populus nigra* (2)	3.5	10.4 ± 0.9	75.1
AA3	40º 39' 53.2'' N 3º 10' 23.9'' E	*A. altissima* (53)	*P. nigra* (1)	12.0	14.5 ± 1.1	97.8
FA1	40º 57' 18.0'' N 2º 55' 20.2'' W	*Fraxinus angustifolia* (15)		6.3	19.9 ± 3.2	100.0
FA2	40º 57' 16.2'' N 2º 55' 50.4'' W	*F. angustifolia* (25)	*Juglans regia* (1) *R. pseudoacacia* (5)*	8.0	15.7 ± 1.7	56.0
FA3	40º 30' 57.3'' N 3º 18' 13.8'' W	*F. angustifolia* (23)	*Morus spp.* (1) *P. alba* (4)* *P. nigra* (3)	3.3	11.0 ± 0.7	56.4
PA1	40º 56' 49.2'' N 2º 56' 19.2'' W	*Populus alba* (11)		11.8	36.0 ± 2.7	100.0
PA2	40º 43' 29.8'' N 3º 9' 21.1'' W	*P. alba* (24)		12.2	24.1 ± 1.7	100.0
PA3	40º 57' 19.3'' N 2º 55' 43.1'' W	*P. alba* (59)		6.8	11.6 ± 0.4	100.0
PA4	40º 36' 43.9'' N 3º 12' 23'' W	*P. alba* (41)		2.9	9.3 ± 0.3	100.0
RP1	40º 56' 48.4'' N 2º 56' 15.7'' W	*Robinia pseudoacacia* (26)	*F. angustifolia* (3)*	4.7	13.4 ± 1.0	94.0
RP2	40º 56' 57.9'' N 2º 56' 1.3'' W	*R. pseudoacacia* (21)	*P. nigra* (1)	1.4	8.5 ± 0.7	74.4
RP3	40º 56' 48.0'' N 2º 56' 17.2'' W	*R. pseudoacacia* (16)	*Juglans regia* (1) *F. angustifolia* (4)*	6.5	18.5 ± 1.6	77.1

*The contribution of these species was summed to that of the dominant species to calculate per-plot monthly/annual input of mass and nutrients.

Litter nutrients

The litter collected during the 1-3 month period of maximum litterfall in 2012 was reserved for nutrient analyses. We only analyzed the litter of the four target species and the main litter fractions (i.e. the "unidentifiable debris" fraction, which was on average 9 ± 2 % of the total litter, was not analyzed because its composition changes over time). We used only the samples collected by hanging and pot traps because they were not in direct contact with the soil,

reducing the risk of fungal colonization. All samples from the same plot, species and plant litter fraction were pooled into a single compound sample and ground with a Cullati mill (0.7 mm particle size). N and P concentration were determined with a segmented flux auto-analyzer (Skalar San^{++}) after digestion with H_2SO_4 and Cu-KSO_4. To calculate N and P inputs per month, we used a single estimate of the N and P concentration of each plant organ for each selected species, then multiplied by the grams of litter of each plant organ supplied by the dominant species, plus any of the additional studied species if present (see Table 1). In each plot, annual N and P inputs were estimated as the sum of N and P inputs in each of the twelve months (June-May).

Soil collection and soil properties

From each plot, in March (spring) and December (autumn) 2012 we collected a mixed soil sample, from the vicinity of each of the four hanging traps. Each mixed sample consisted of three soil cores (7 x 7 cm x 11 cm deep) collected and pooled together. Soil samples were carried to the lab in polyethylene bags, spread on filter paper and allowed to air dry. Afterwards, soil samples were sieved with a 1 mm mesh, homogenized, and kept at 4 ºC until analysis.

To analyze total N and P concentrations, 0.5 g of soils were digested with H_2SO_4 and Cu–KSO_4. The digested solution was analyzed with a segmented flux autoanalyzer (Skalar San^{++}). We determined soil OM following a modification of the method described by Nelson and Sommers (1996). We weighed 5-10 g of soil previously dried at 105 °C for 48 h. Then the soil was burned at 400 °C for 2 h, cooled in a desiccator to room temperature and reweighed. OM (%) was calculated as the proportion of weight loss. We measured pH in a slurry of 20 g of soil with 40 ml of deionized water using a pH meter (micropH 2001, Crison Instruments, Barcelona, Spain) (Allen et al. 1974).

Statistical analysis

Since TTBA (m^2) varied across plots, and the litter mass and nutrient inputs at plot level tend to increase with TTBA (Online resource 1), we expressed all litterfall variables per unit of surface area and TTBA ($g\ m^{-4}$) to make them comparable across plots. Similarly, we expressed soil N, P and OM per TTBA unit (m^2), since they also tended to increase with TTBA (Online resource 2). This approach is consistent with other studies that found significant positive correlations between litterfall and tree size (Negash and Starr 2013) or between litterfall and TTBA (Williams and Wardle 2007; González et al. 2010).

For each plant fraction, differences across species in N and P concentrations were assessed by one-way ANOVA followed by Tukey HSD post-hoc test. Data were log or square-root transformed when appropriate to attain normality and/or homocedasticity. To assess differences in the percentage of each litter fraction, the total and per-fraction annual litter mass and N and P inputs across forest types and years, we used linear mixed models with forest type and year as fixed factors and plot nested within forest type as a random factor. After three months of sampling, one *P. alba* plot (PA3, Table 1) had to be moved to another location because of a timber harvest in the original plot. This plot therefore is missing data for the first three months. The model using the interaction term between year and forest type did not significantly differ ($P > 0.05$) from the model without the interaction, so we did not include the interaction.

To compare differences in total and per-fraction monthly input of litter mass, N and P produced by the dominant species, we used linear mixed models with dominant species and month as fixed factors and plot nested within dominant species as a random factor. We concluded that the temporal patterns of litterfall, N or P varied across species when the interaction term "dominant species x month" was significant. The model using the interaction term between

year and dominant species did not significantly differ ($P > 0.05$) from the model without the interaction, so we did not include this interaction.

Differences in soil N, P and OM and pH across forest types were analysed using generalized linear mixed models (family = gamma) (Bolker et al. 2009), with forest type and season (March vs. December) as fixed factors and plot nested within forest type as a random factor. Tukey´s all pairwise comparisons were conducted in R package with the *glht* function in the software package *multcomp* (Hothorn et al. 2008). We used R package 2.14.0 (R Development Core Team, 2011) for all data analyses and the level of significance considered was always $P < 0.05$).

Results

Annual quantity and dynamics of litterfall

In the study plots, the different litter fractions included flowers (6 % of the total annual litter), fruits (10 %), leaves (69 %), bark and branches (16 %), and unidentifiable debris (9 %), with no significant differences among forest types ($F_{3;21} < 0.83$, $P > 0.05$ in all cases).

The peak of flower abscission in the exotic trees occurred in June, 2-3 months later than in the natives (April for *P. alba* and March-April for *F. angustifolia*) (Fig. 2 a). *P. alba* fruits fell in May and June, but the remaining species extended fruit dispersal throughout the year (Fig. 2 b). The shedding of bark and branches did not show any clear temporal pattern (Fig. 2 c). All species showed their main peak of litterfall during autumn (October-December), coinciding with the period of leaf shedding of deciduous trees (Fig. 2 d and e). However, exotic species showed extra litterfall peaks, which were absent in the natives (significant interaction between dominant species and month, $F_{69, 243} = 2.13$, $P < 0.0001$) (Fig. 2 e). *R. pseudoacacia* showed additional peaks in summer (August 2011 and September 2011; Fig. 2 e) corresponding to a

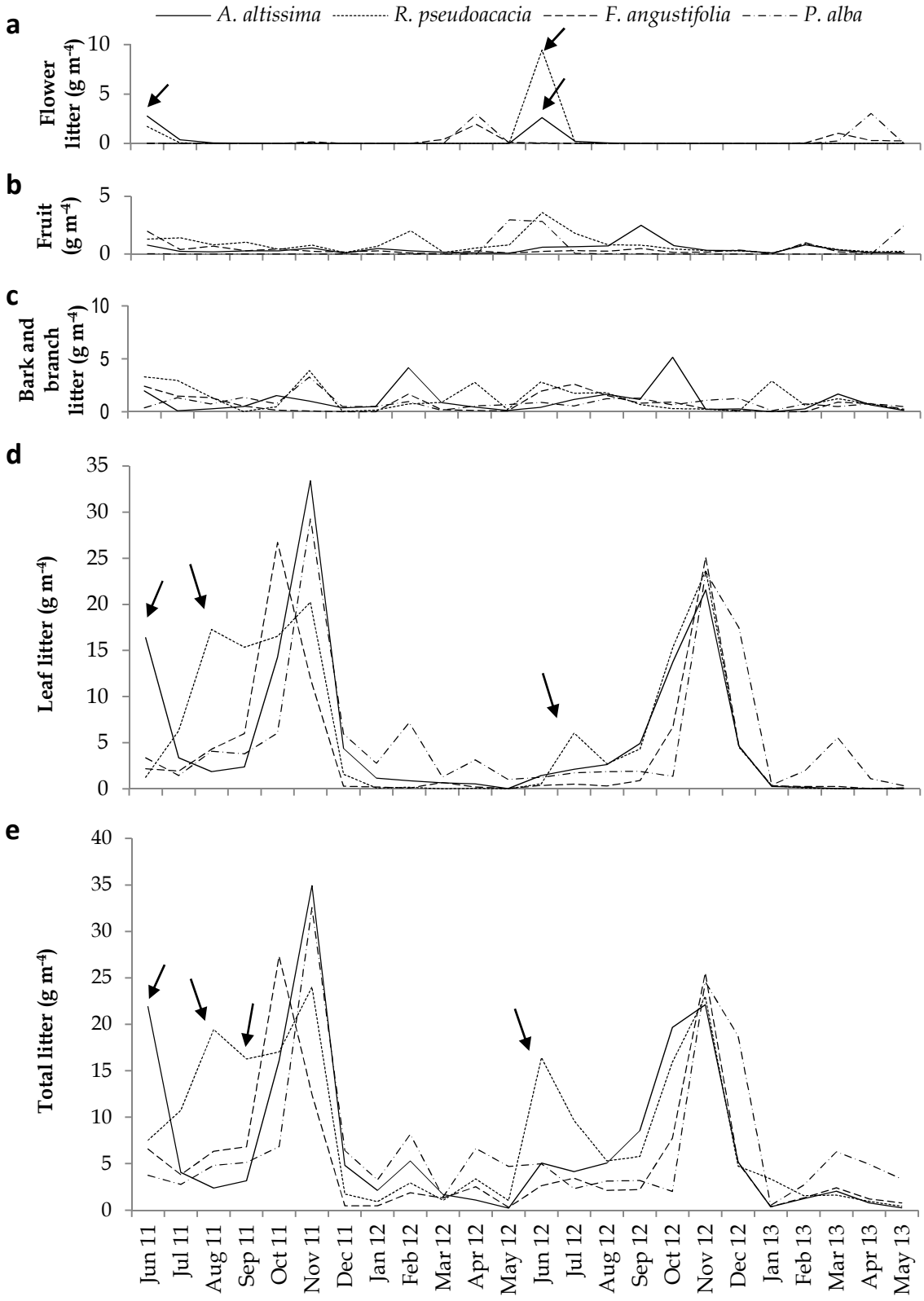


Figure 2. Mean flower (**a**), fruit (**b**), bark and branch (**c**), leaf (**d**) and total litter (**e**), provided by the dominant species (*Ailanthus altissima*, *Robinia pseudoacacia*, *Fraxinus angustifolia* and *Populus alba*) in each month (from Jun 2011 to May 2013) by collection surface and total tree basal area (g m^{-4}). Arrows represent litterfall peaks from the invasive species almost absent in the natives. Standard errors were not shown for clarity.

late-summer leaf abscission (Fig. 2 d) and another extra peak in late spring (June 2012) due to the shedding of flowers and fruits (Fig. 2 a and b). *A. altissima* also showed a late-spring peak (June 2011) due to early leaf shedding (Fig. 2 d and e). Flowers represented a large proportion of the litter at their time of maximum floral abscission; flowers made up 34 % of the total litter for *A. altissima*, 43% for *R. pseudoacacia*, 56 % for *F. angustifolia* and 48% for *P. alba* during the month of maximum flower fall for each tree species. The proportion of total annual litter fallen in summer 2011 (July to September) was 47 % in forests invaded by *R. pseudoacacia* but only 24 % and 12 % in forests dominated by *F. angustifolia* and *P. alba*, respectively. The late-spring peaks of *A. altissima* and *R. pseudoacacia* accounted for 21 and 16 % of total annual litter, respectively. By contrast, this proportion was less than 10 % in non-invaded forests.

There was no significant difference among forest types either in the total annual litter mass or in the annual litter by fractions (flowers, fruits, leaves, bark and branches), but forests invaded by *R. pseudoacacia* showed a non-significant tendency to produce more litter than the other forests (1.4-1.7 times more; Table 2). The annual leaf litter was significantly greater in the first than in the second year in all forest types (Table 2).

Table 2. Mean (± SE) total and per fraction (flowers, fruits, leaves and bark and branches) annual litter (g m^{-4} $year^{-1}$) collected in different forest types (*Ailanthus altissima*, *Robinia pseudoacacia*, *Fraxinus angustifolia* and *Populus alba* forest) and the F and *P* values for linear mixed models assessing the effects of forest type and year on annual litterfall variables.

	Litter production (g m^{-4} $year^{-1}$)								**Fixed effects**			
	Exotics				**Natives**							
	A. altissima		*R. pseudoacacia*		*F. angustifolia*		*P. alba*		**Forest type**		**Year**	
Litter fractions	*Year 1* **(n = 3)**	*Year 2* **(n = 3)**	*Year 1* **(n = 3)**	*Year 2* **(n = 3)**	*Year 1* **(n = 3)**	*Year 2* **(n = 3)**	*Year 1* **(n = 3)**	*Year 2* **(n = 4)**	F	*P*	F	*P*
Flowers	3.2 ± 1.5	2.8 ± 1.2	12.3 ± 8.0	8.2 ± 3.5	3.5 ± 0.4	3.0 ± 0.4	3.9 ± 1.6	3.8 ± 2.8	1.30	0.334	2.05	0.180
Fruits	3.7 ± 1.7	11.2 ± 6.6	14.0 ± 2.7	17.1 ± 5.0	6.1 ± 3.4	5.5 ± 2.3	4.0 ± 2.5	5.6 ± 2.6	2.08	0.173	1.69	0.220
Leaves	68 ± 26	47 ± 17	87 ± 32	66 ± 20	60 ± 20	40 ± 12	64 ± 31	61 ± 29	0.29	0.834	22.76	**0.0001**
Bark and branches	11.9 ± 4.6	12.4 ± 5.4	14.8 ± 9.1	14.5 ± 6.1	7.6 ± 2.4	8.4 ± 3.2	13.1 ± 2.2	10.7 ± 0.5	0.40	0.758	0.14	0.713
Total litter	80 ± 22	78 ± 19	113 ± 37	114 ± 31	73 ± 23	64 ± 14	76 ± 32	84 ± 27	0.55	0.661	0.01	0.919

Significant *P*-values are indicated in bold.

Litter nutrients

P. alba litter showed low N and P concentrations in most of the fractions (Tables 3 and 4). The remaining species had similar N concentrations in their different fractions, except *R. pseudoacacia* fruits, which had the highest N and P concentrations (Table 3 and 4). *F. angustifolia* had the highest P concentration in bark and branches of all studied species, while *Ailanthus altissima* had the highest P concentration in flowers (Table 4).

Table 3. Mean (± SE) of nitrogen (N) concentration contained in flowers, fruits, leaves, and bark and branches of each species (*Ailanthus altissima*, *Robinia pseudoacacia*, *Fraxinus angustifolia* and *Populus alba*) assessed at the time of the maximum abscission in 2012 for each plot.

Nitrogen (%)				
	Exotics		Natives	
Litter fractions	*A. altissima* **(n = 3)**	*R. pseudoacacia* **(n = 3)**	*F. angustifolia* **(n = 3)**	*P. alba* **(n = 4)**
Flower	4.90 ± 0.52 ^{a}	4.95 ± 0.13 ^{a}	5.09 ± 0.40 ^{a}	2.33 ± 0.61 ^{b}
Fruits	4.80 ± 0.31 ^{b}	11.70 ± 0.27 ^{a}	4.05 ± 0.25 ^{b}	2.36 ± 0.35 ^{c}
Leaves	3.64 ± 0.35 ^{a}	3.86 ± 0.34 ^{a}	3.73 ± 0.31 ^{a}	1.42 ± 0.14 ^{b}
Bark and branches	1.78 ± 0.17 ^{ab}	2.29 ± 0.10 ^{ab}	2.71 ± 0.60 ^{a}	0.86 ± 0.17 ^{b}

Different letters in a row indicate significant differences among species for N ($P < 0.05$, one-way ANOVA, Tukey HSD test)

Table 4. Mean (± SE) of phosphorus (P) concentration contained in flowers, fruits, leaves, and bark and branches of each species (*Ailanthus altissima*, *Robinia pseudoacacia*, *Fraxinus angustifolia* and *Populus alba*) assessed at the time of the maximum abscission in 2012 for each plot.

Phosphorus (%)				
	Exotics		Natives	
Litter fractions	*A. altissima* **(n = 3)**	*R. pseudoacacia* **(n = 3)**	*F. angustifolia* **(n = 3)**	*P. alba* **(n = 4)**
Flower	0.70 ± 0.11 ^{a}	0.45 ± 0.01 ^{ab}	0.44 ± 0.02 ^{ab}	0.29 ± 0.08 ^{b}
Fruits	0.72 ± 0.08 ^{b}	1.29 ± 0.14 ^{a}	0.61 ± 0.09 ^{b}	0.37 ± 0.08 ^{b}
Leaves	0.28 ± 0.04 ^{a}	0.14 ± 0.03 ^{a}	0.49 ± 0.25 ^{a}	0.15 ± 0.04 ^{a}
Bark and branches	0.14 ± 0.04 ^{ab}	0.10 ± 0.03 ^{b}	0.33 ± 0.07 ^{a}	0.13 ± 0.05 ^{ab}

Different letters in a row indicate significant differences among species for P ($P < 0.05$, one-way ANOVA, Tukey HSD test)

The temporal pattern of N and P input differed among species (significant interaction between forest type and month; $F_{69,243} = 2.22$, $F_{69,243} = 2.04$ for N and P inputs, respectively; $P < 0.0001$). The additional summer leaf peak of *R. pseudoacacia* resulted in an additional peak of N but not of P (Fig. 3 a and b), because *R. pseudoacacia* leaf litter had low P concentration (Table 4). The additional late-spring peaks of *A. altissima* and *R. pseudoacacia* resulted in additional peaks of N and P (Fig. 3 a and b).

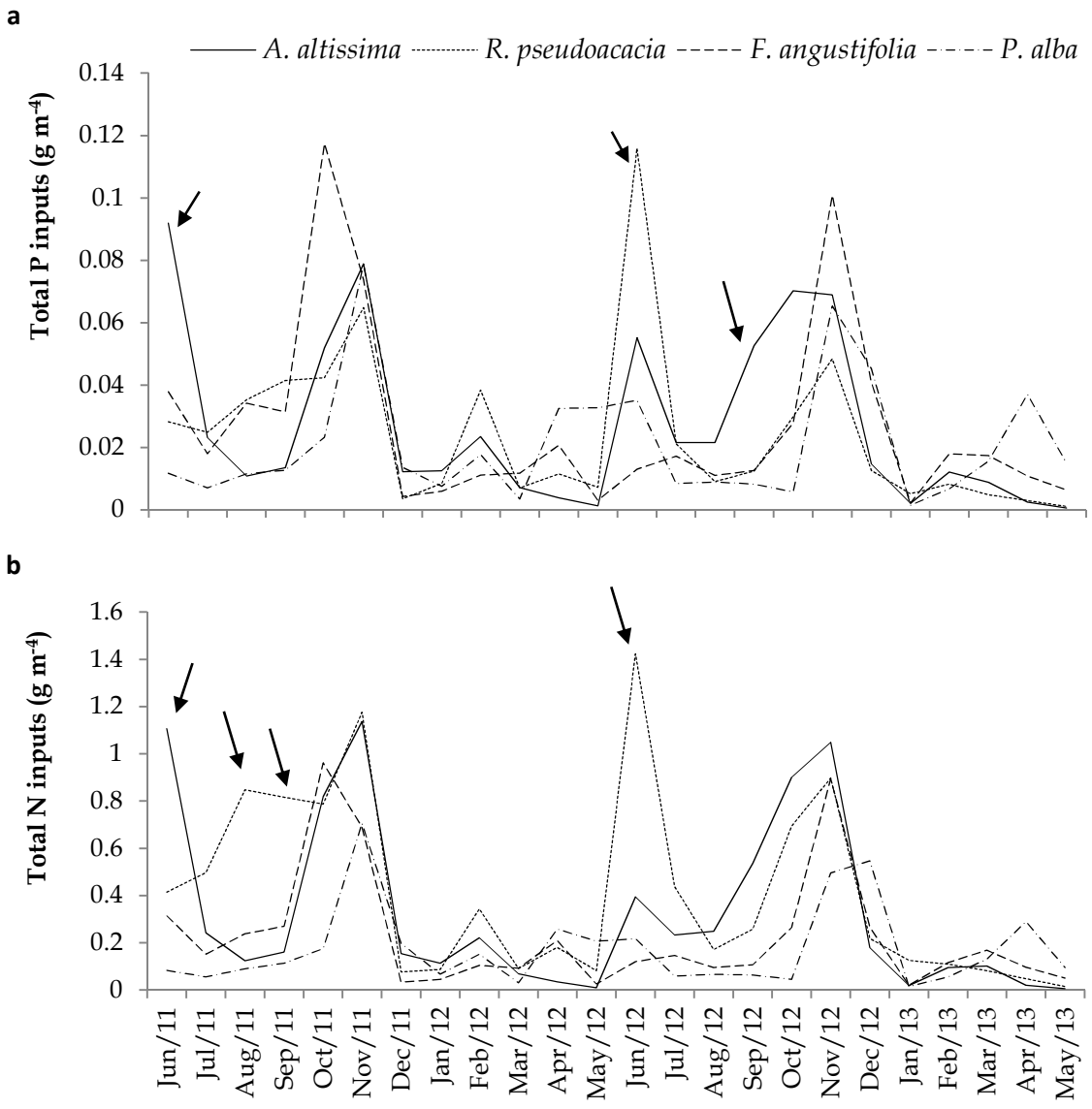


Figure 3. Mean total phosphorus (P) (**a**) and nitrogen (N) (**b**) inputs estimated from total litter produced in each community (*Ailanthus altissima, Fraxinus angustifolia, Populus alba and Robinia pseudoacacia*) per month (from Jun 2011 to May 2013). Arrows represent litterfall peaks from the invasive species almost absent in the natives. Standard errors are not shown for clarity.

Total N and P inputs did not significantly differ among forest types because of the high variability, although average N inputs were 1.7-2.2 times higher in invaded than in non-invaded forests (Tables 5 and 6). Annual N inputs from fruits were marginally different among forest types, tending to be greater in forests invaded by *R. pseudoacacia* than in the other forests (Table 5). However, annual N inputs from other litter fractions did not significantly differ among forest types. Due to greater quantities of leaf litter, both N and P inputs from leaf litter were significantly greater in the first year than in the second year (Table 5 and 6).

Soil properties

Forests invaded by *R. pseudoacacia* had the highest OM and soil N concentrations, while *P. alba* forests had the lowest soil OM, N and P (Fig. 4). *A. altissima* and *F. angustifolia* forests showed intermediate and similar values of soil OM and N (Fig. 4 a and b). Soil OM and N were greater in autumn than in spring (Fig. 4 a and b). However, soil P tended to decrease from spring to autumn in *A. altissima* and *F. angustifolia* forests and increase in *P. alba* and *R. pseudoacacia* forests (Fig. 4 c). Soil pH did not significantly differ among forest types, but it was higher in autumn than in spring in all forests except in *F. angustifolia* forests, where it was higher in spring than in autumn (Fig. 4 d).

Table 5. Mean (± SE) nitrogen (N) inputs from total and per fraction (flowers, fruits, leaves, and bark and branches) annual litter (g m^{-4} $year^{-1}$) collected in different forest types (dominated by *Ailanthus altissima*, *Robinia pseudoacacia*, *Fraxinus angustifolia* and *Populus alba*) and the F and *P* values for linear mixed models assessing the effects of forest type and year on annual N input variables.

	Nitrogen inputs (g m^{-4} $year^{-1}$)								**Fixed effects**			
	Exotics				Natives							
	A. altissima		*R. pseudoacacia*		*F. angustifolia*		*P. alba*		**Forest type**		**Year**	
Litter	*Year 1*	*Year 2*	*Year 1*	*Year 2*	*Year 1*	*Year 2*	*Year 1*	*Year 2*				
fractions	(n = 3)	(n = 3)	(n = 3)	(n = 4)	(n = 3)	(n = 3)	(n = 3)	(n = 4)	F	*P*	F	*P*
Flowers	0.17 ± 0.10	0.15 ± 0.08	0.09 ± 0.06	0.48 ± 0.25	0.12 ± 0.06	0.09 ± 0.05	0.12 ± 0.08	0.12 ± 0.12	0.89	0.481	0.50	0.496
Fruits	0.17 ± 0.08	0.33 ± 0.21	0.36 ± 0.04	0.37 ± 0.06	0.20 ± 0.09	0.14 ± 0.11	0.10 ± 0.07	0.14 ± 0.07	3.57	0.060	0.69	0.423
Leaves	1.79 ± 0.9	1.14 ± 0.51	1.53 ± 0.48	1.12 ± 0.30	1.25 ± 0.24	0.90 ± 0.11	0.88 ± 0.41	0.71 ± 0.26	0.93	0.464	58.68	**< 0.001**
Bark and branches	0.21 ± 0.08	0.24 ± 0.11	0.31± 0.23	0.26 ± 0.12	0.20 ± 0.13	0.28 ± 0.22	0.08± 0.01	0.08 ± 0.01	0.79	0.530	0.04	0.836
Total litter	2.33 ± 0.75	1.86 ± 0.67	2.58 ± 0.55	2.30 ± 0.69	1.53 ± 0.21	1.15 ± 0.07	1.18 ± 0.34	1.06 ± 0.18	2.66	0.112	10.01	**0.009**

Significant *P*-values are indicated in bold.

Table 6. Mean (± SE) total and per fraction (flowers, fruits, leaves, and bark and branches) annual litter (g m^{-4} $year^{-1}$) collected in different forest types (dominated by *Ailanthus altissima*, *Robinia pseudoacacia*, *Fraxinus angustifolia* and *Populus alba*) and the F and *P* values for linear mixed models assessing the effects of forest type and year on annual P input variables.

	Phosphorus inputs (g m^{-4} $year^{-1}$)								**Fixed effects**			
	Exotics				Natives							
	A. altissima		*R. pseudoacacia*		*F. angustifolia*		*P. alba*		**Forest type**		**Year**	
Litter fractions	*Year 1* (n = 3)	*Year 2* (n = 3)	*Year 1* (n = 3)	*Year 2* (n = 4)	*Year 1* (n = 3)	*Year 2* (n = 3)	*Year 1* (n = 3)	*Year 2* (n = 3)	F	*P*	F	*P*
Flowers	0.026 ± 0.015	0.023 ± 0.012	0.008 ± 0.006	0.041 ± 0.020	0.012 ± 0.005	0.008 ± 0.004	0.015 ± 0.011	0.016 ± 0.015	1.05	0.416	0.10	0.762
Fruits	0.025 ± 0.014	0.045 ± 0.026	0.030 ± 0.004	0.031 ± 0.004	0.032 ± 0.015	0.023 ± 0.018	0.016 ± 0.011	0.024 ± 0.013	0.73	0.559	0.69	0.425
Leaves	0.113 ± 0.041	0.072 ± 0.023	0.049 ± 0.010	0.036 ± 0.006	0.160 ± 0.095	0.117 ± 0.069	0.106 ± 0.050	0.078 ± 0.033	0.39	0.761	56.23	**< 0.001**
Bark and branches	0.018 ± 0.010	0.022 ± 0.014	0.012 ± 0.008	0.010 ± 0.004	0.023 ± 0.013	0.030 ± 0.022	0.009 ± 0.002	0.012 ± 0.004	0.09	0.964	0.00	0.962
Total litter	0.18 ± 0.03	0.16 ± 0.04	0.15 ± 0.02	0.14 ± 0.02	0.18 ± 0.08	0.14 ± 0.03	0.15 ± 0.04	0.13 ± 0.02	0.26	0.854	2.24	0.163

Significant *P*-values are indicated in bold.

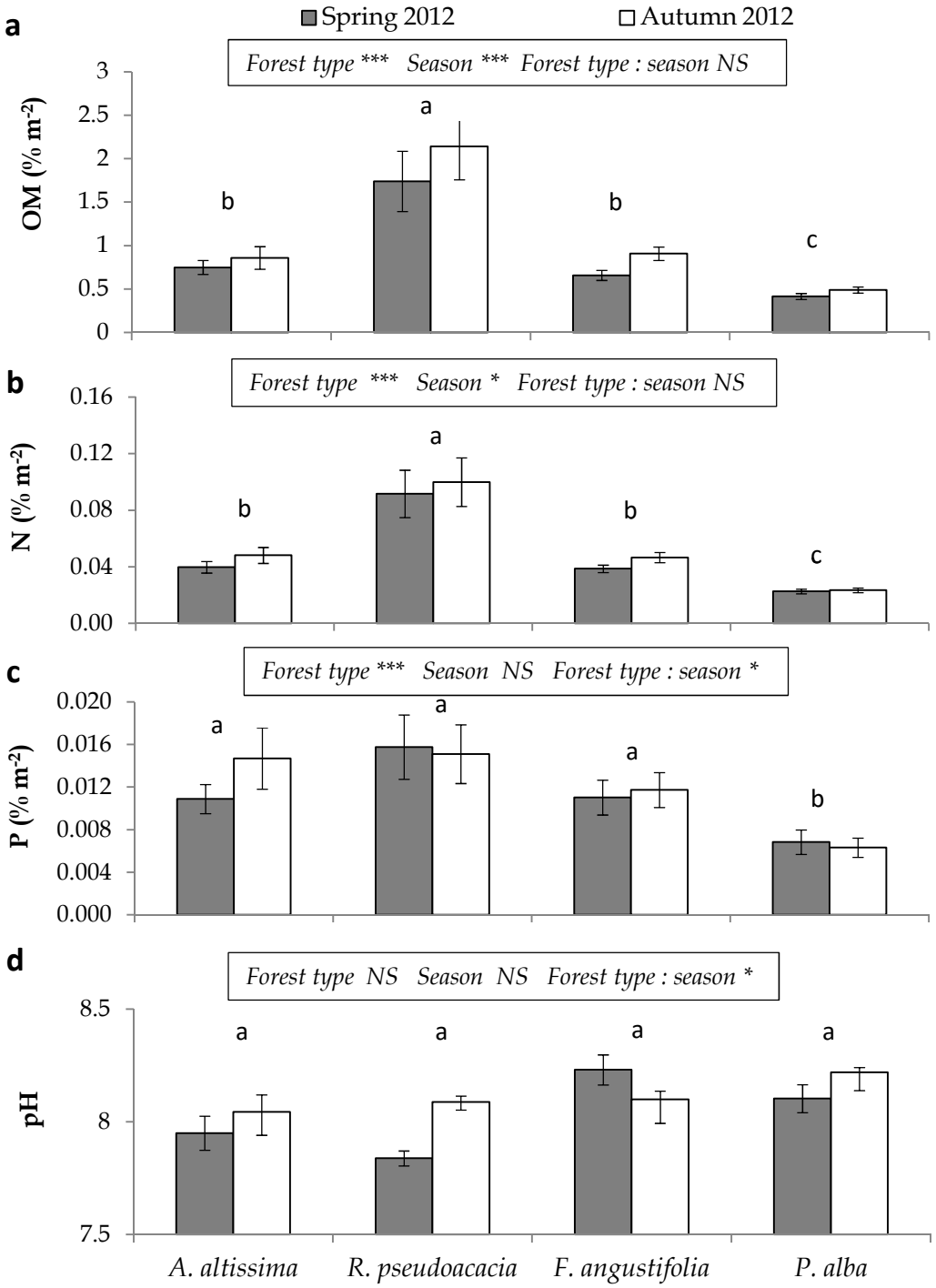


Figure 4. Mean (± SE) soil organic matter (OM) (**a**), nitrogen (N) (**b**) and phosphorus (P) (**c**), concentration per unit of total tree basal area (m²), and pH (**d**) in two seasons (spring and autumn 2012) in *Ailanthus altissima*, *Robinia pseudoacacia*, *Fraxinus angustifolia* and *Populus alba* forests. Different letters mean significant differences among forest types (Tukey HSD test). The significance of forest type, season and their interaction is shown in each chart (NS: No significant; * $P < 0.05$; ** $P < 0.01$: *** $P < 0.001$).

Discussion

In our study, forests invaded by *R. pseudoacacia* had the greatest soil OM, N and P, while native forest dominated by *P. alba* showed the lowest values. This result is consistent with patterns of the N and P content of litter, which was the greatest in *R.pseudoacacia* and the lowest in *P. alba* in most litter fractions, even though we failed to find differences in total N or P inputs or in the quantity of litter among forest types. The capacity of *R. pseudoacacia* to fix N, and its low N use efficiency (González-Muñoz et al. 2013) may explain the higher N concentration of some of its senescent tissues. Additions of N-rich litter from trees have been reported to increase soil N (Johansson 1995). Litter from different plant organs of both invasive species had higher N content than that of *P. alba*, which likely led to greater soil N in invaded than in *P. alba* soils. In addition, greater quantities of litter usually increase total soil N and OM (Martin et al. 2009), and we found a (non-significant) trend for *R. pseudoacacia* to produce more annual litter (up to 44% more) and N inputs (up to 56% more) than the remaining species (Table 2 and 5). This trend likely reflects the high growth rates and poor N use efficiency of *R. pseudoacacia* (González et al. 2010; Cierjacks et al. 2013; González-Muñoz et al. 2013; Castro-Díez et al. 2014c).

Soil OM depends not only on the quantity and quality of litter inputs, but also on the decomposability of the litter, the root exudates of organic carbon, the persistence of the different OM compounds, and the soil microbial biomass and activity (Schmidt et al. 2011). In the case of *R. pseudoacacia*, previous studies in Central Spain have pointed to the potential of *R. pseudoacacia* to accumulate recalcitrant OM and N in the soil due to its high lignin content and the consequent slower leaf litter decomposition than coexisting native species (Castro-Díez et al. 2009, 2012). Moreover, flowers from the exotic species fell in June, at the beginning of the dry season (2-3 months later than those from the natives), when microbial and plant activity declines due to water shortage. These arid conditions may slow down decomposition and the uptake of the

derived nutrients, enlarging the period when organic matter and nutrients remains in the soil.

The summer and late-spring inputs from the exotic species were mostly due to leaf abscission and probably reflect the adjustment of these species to reduce water loss during the arid period. Neither *A. altissima* nor *R. pseudoacacia* evolved in regions with strong summer water stress, and they may have fewer adaptations than native species to tolerate water shortage. In fact, the time of the additional leaf litter peaks displayed by exotics match the times of lowest precipitation and highest temperature (Fig. 1 and 2 d). The leaf litter that falls in summer could decay more slowly, due to water shortage during the summer. This would enlarge the time when organic nutrients remain in the soil and contribute to the increment in soil nutrients and organic matter in invaded forests (Kirschbaum 2010).

Given that N and P concentration may vary in plant tissues across the year, measuring nutrients only at the peak of each fraction fall may introduce errors into the calculation of the annual input of nutrients in the soil, particularly in the case of *R. pseudoacacia* and *A. altissima*, which showed early peaks of leaf abscission (Fig. 2). Given that pre-senescent leaf litter can have greater nutrient content due to a more limited nutrient resorption (Killingbeck 1996), our calculation may have underestimated the amount of N and P input to the soil in the sites invaded by the two exotic species. In the remaining species, we believe that the potential error was lower because 1) the fraction of flowers, fruits and leaves that fell out of the period of maximum fall was lower; 2) *P. alba* leaves have been found to have similar N concentrations whether they fall in summer or autumn (González-Muñoz et al. 2013), and 3) annual changes in N and P of branches of Mediterranean phanaerophytes in general have been found to be quite low (Milla et al. 2005).

The additional litterfall peaks produced in late spring by the invasive species may generate additional soil litter layers, which may act as physical barriers for germination, establishment and growth of species that shed their

seeds in spring and do not form soil seed banks (Facelli and Picket 1991). This is the case for *P. alba,* whose seeds disperse in May-June and are viable for only a few weeks (Prada and Arizpe 2008). Germination of small seeds, such as those of *P. alba,* may be more hindered by litter layers than that of bigger seeds, such as those of *A. altissima* and *R. pseudoacacia* (Facelli and Picket 1991; Seiwa and Kikuzawa 1996). Therefore, the re-colonization of invaded forests by the native *P. alba* could be hindered by the accumulation of spring litter layers from exotic trees. At the same time, seed germination of these exotics species could be less affected by their own summer and spring litter since they shed their fruits throughout the year (Castro-Díez et al. 2014c).

Additional litter inputs with different quality from invasive species may affect abundance and composition of detritivore communities, which are in synchrony with litterfall processes (Takeda 1987; Ponge et al. 1993). Some detritivores may not be adapted to feed on recalcitrant leaf litter (Bastow et al. 2008), such as that of *R. pseudoacacia*, which may lead to a longer persistence of *R. pseudoacacia* litter layers. However, the abundance of some arthropod species may also increase with additions of high quality litter (Cesarz et al. 2007), such as *A. altissima* litter with its high nutrient content and rapid decomposition (Castro-Díez et al. 2009, 2012). In fact, soils invaded by *A. altissima* host altered soil arthropod communities compared to non-invaded soils (Gutiérrez-López et al. 2014; Motard et al. 2015)

Conclusions

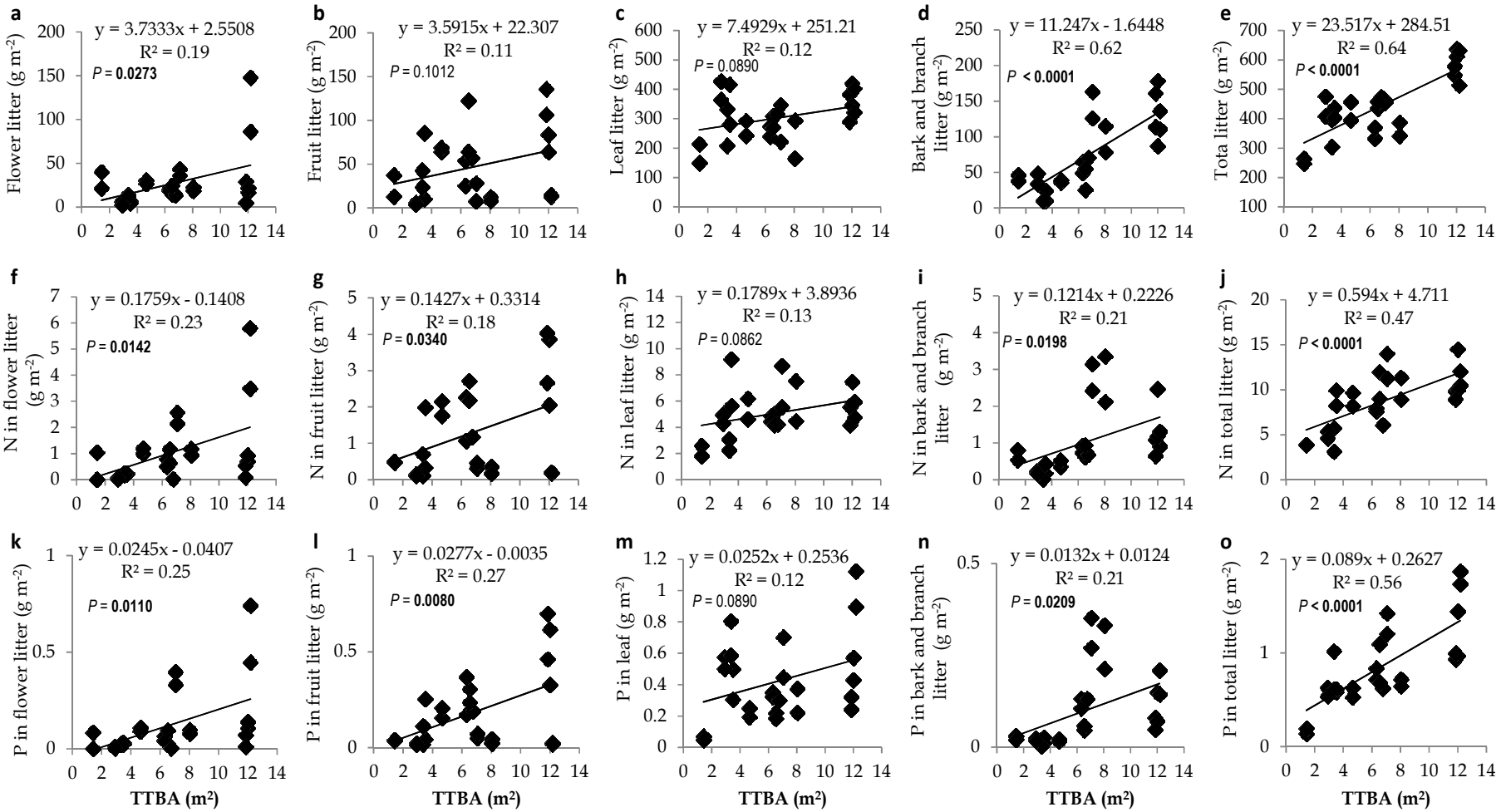
In conclusion, our study highlight the potential of the invasive trees, *A. altissima* and *R. pseudoacacia*, to alter soil properties even when we did not find significant differences in the annual quantities of litterfall and nutrient inputs between invaded and native forests. *R. pseudoacacia* tended to produce more annual litter and N inputs than the remaining species, likely due to its high growth rate and poor N use efficiency. Forests invaded by *R. pseudoacacia* had higher concentrations of soil nutrients and OM than native riparian forests.

These soil variables were also elevated in *A. altissima* forests, but only when compared with *P. alba* forests. Finally, invasive trees altered litterfall dynamics by producing additional litterfall peaks and N and P inputs in summer and late-spring, which may modify soil nutrients, the activity of detritivores, as well as seed germination success.

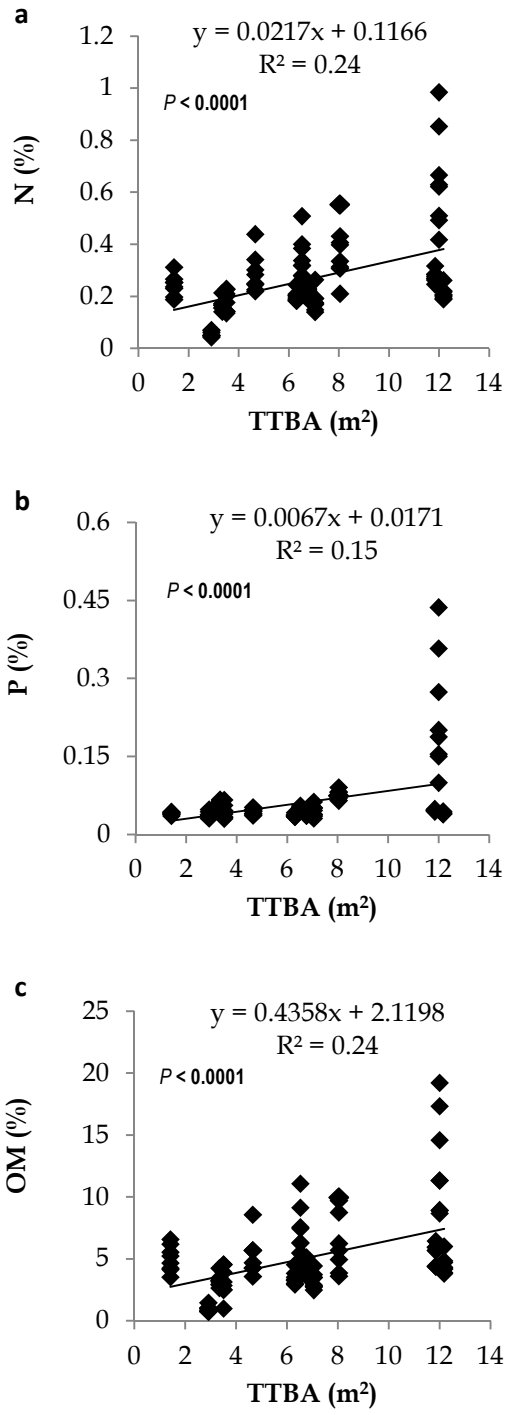
Acknowledgements

This study was supported by the projects CGL2011-16388/BOS of the *Ministerio de Economía y Competitividad* of Spain and POII10-0179-4700 of the Junta de Comunidades de Castilla-La Mancha. Silvia Medina Villar was supported by a grant from the *Ministerio de Economía y Competitividad* of Spain (FPI fellowship, BES-2011-048379). We are grateful to the support of REMEDINAL3-CM MAE-2719 (Comunidad de Madrid), to Guillermo Valle-Torres and Mónica Otero for their help with field and lab work and to Asier Herrero for climatic data.

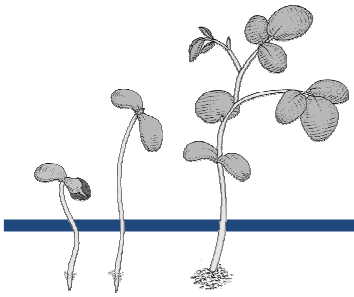
Supplementary Information



Online resource 1. Linear regressions among total tree basal area (TTBA) (m²) and annual flower (**a**), fruit (**b**), leaf (**c**), bark and branch (**d**) and total (**e**) litter, nitrogen (N) in flower (**f**), fruit (**g**), leaf (**h**), bark and branch (**i**) and total litter (**j**), phosphorous (P) in flower (**k**), fruit (**l**), leaf (**m**), bark and branch (**n**) and total litter (**o**) produced in the different forests (invaded by *A. altissima*, invaded by *R. pseudoacacia* and dominated by *P. alba* and dominated by *F . angustifolia*).



Online resource 2. Linear regressions among total tree basal area (TTBA) (m^2) and soil nitrogen (N) (**a**) phosphorus (P) (**b**) and organic matter (OM) (**c**) in the different forests (invaded by *Ailanthus altissima*, invaded by *Robinia pseudoacacia* and dominated by *Populus alba* and dominated by *Fraxinus angustifolia*).



Capítulo 3

Impacts of the alien trees *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. on soil nutrients and microbial communities

Oh Coimbra do Mondego
e dos amores que eu lá tive
quem não te viu anda cego
quem te não ama não vive

Do Choupal até à Lapa
foi Coimbra meus amores
a sombra da minha capa
deu no chão abriu em flores

José Afonso

Este capítulo reproduce el texto del siguiente manuscrito:

This chapter reproduces the text of the following manuscript:

Medina-Villar S, Rodríguez-Echeverría S, Lorenzo P, Alonso A, Pérez-Corona E, Castro-Díez P (2016) Impacts of the alien trees *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. on soil nutrients and microbial communities. Soil Biology and Biochemistry 96: 65-73. doi: 10.1016/j.soilbio.2016.01.015

Fotografía: Vegetación de ribera del Río Henares cerca de Chiloeches (Provincia de Guadalajara, España). Mancha de vegetación dominada por el árbol nativo, *Populus alba* (parte izquierda de la foto), junto a una mancha dominada por el árbol invasor *Ailanthus altissima* (parte izquierda de la foto).
Por: Silvia Medina Villar

Photography: Riparian vegetation of the Henares River near to Chiloeches (Gudalajara province, Spain). Vegetation patch dominated by the native tree, *Populus alba* (left part of the photograph), next to a patch dominated by the invasive tree *Ailanthus altissima* (right part of the photograph).
By: Silvia Medina Villar

El dibujo de los encabezados está modificado de Cierjacks et al. (2013).

Picture of headers is a modification from Cierjacks et al. (2013)

Resumen

Impactos de los árboles invasores *Ailanthus altissima* (Mill.) Swingle y *Robinia pseudoacacia* L. en los nutrientes y en la comunidad microbiana del suelo

Ailanthus altissima Mill. (Swingle) y *Robinia pseudoacacia* L. son dos especies de árboles invasores en zonas riparias del Centro de España. Nuestro objetivo fue comprobar si las propiedades del suelo, las tasas de mineralización de nutrientes y la comunidad edáfica de bacterias de bosques de ribera dominados por la especie nativa *Populus alba* L. podían ser alteradas por la presencia de *A. altissima* o *R. pseudoacacia.* En otoño del 2011 y primavera del 2012 llevamos a cabo un muestreo de suelo en campo, en tres sitios donde se seleccionaron pares de árboles invasores y nativos. Además, en un experimento de invernadero (EI), se cultivaron semillas de *A. altissima*, *R. pseudoacacia* y *P. alba* en un suelo recogido de una zona nativa, donde crecieron durante 6 meses. Cuantificamos la materia orgánica (MO), el nitrógeno (N), el fósforo (P), el nitrato (NO_3^-–N), el amonio (NH_4^+–N), el pH, las tasas potenciales netas de amonificación y nitrificación, la actividad fosfomonoesterasa (PME), y la comunidad de bacterias tanto en los suelos del muestreo de campo como en los del EI. Ambos resultados mostraron la capacidad de *A. altissima* para disminuir el N total del suelo y de *R. pseudoacacia* para aumentar el N mineral del suelo. En el campo, los suelos invadidos tenían mayor concentración de NO_3^-–N que los suelos de *P. alba*. Además, los suelos de *R. pseudoacacia* presentaron mayor actividad PME, N total y tasa de amonificación neta mientras que los suelos de *A. altissima* mostraron menor MO, NH_4^+–N, tasas netas de nitrificación y de mineralización del N total que los suelos de *P. alba*. Solo se encontraron diferencias en la composición de la comunidad de bacterias del suelo en las muestras de campo, siendo más evidentes las diferencias entre los suelos de *A. altissima* y *P. alba*, que entre los de *R. pseudoacacia* y *P. alba*. La capacidad de *R. pseudoacacia* para aumentar la concentración de N mineral puede deberse a la

fijación de N_2 en sus raíces, mientras que el potencial de *A. altissima* para disminuir el N total del suelo puede ser atribuido a cambios en el balance entre las entradas y pérdidas de N en el suelo. Aunque los resultados del EI indicaron que los árboles invasores pueden cambiar algunas características del suelo durante etapas tempranas de establecimiento, el mayor número de impactos encontrados en el estudio de campo sugiere que varias propiedades del suelo, como la composición de la comunidad de bacterias, necesitan más tiempo desde la invasión para ser alteradas.

Palabras clave: comunidad de bacterias; DGGE; fosfomonoesterasa; nitrato; nitrógeno; plantas exóticas.

Abstract

Impacts of the alien trees *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. on soil nutrients and microbial communities

Ailanthus altissima (Mill.) Swingle and *Robinia pseudoacacia* L. are two aggressive invasive trees in riparian areas in Central Spain. We aim to test whether soil properties, nutrient mineralization rates and soil bacterial communities of riparian forest dominated by the native *Populus alba* L. can be altered by the presence of *A. altissima* or *R. pseudoacacia.* In autumn 2011 and spring 2012 we conducted a field soil sampling in three sites where invasive and native trees were paired. In addition, in a 6-month greenhouse experiment (GHE), we grew *A. altissima*, *R. pseudoacacia* and *P. alba* from seeds in a soil collected from a native area. We quantified soil organic matter (OM), nitrogen (N), phosphorous (P), nitrate (NO_3^-–N), ammonium (NH_4^+–N), pH, potential net ammonification and nitrification rates, phosphomonoesterase (PME) activity and the composition of soil bacterial community in soils from the field study and from the GHE. Both the field and the GHE results showed the capability of *A. altissima* to decrease soil total N and of *R. pseudoacacia* to increase soil mineral N. In the field, all invaded soils had greater NO_3^-–N than *P. alba* soils. *R. pseudoacacia* field soils had greater PME activity, total N and net ammonification rates while *A. altissima* soils had lower OM, NH_4^+–N, net nitrification and total N mineralization rates than those of *P. alba*. Differences in the composition of soil bacterial communities were only found in the field, being more evident between *A. altissima* and *P. alba* than between *R. pseudoacacia* and *P. alba* field soils. Symbiotic N_2 fixation could explain the capability of *R. pseudoacacia* to increase soil mineral N, while the potential of *A. altissima* to decrease total soil N may be attributed to changes in the balance between N input and losses from the soil. Although the GHE results indicated that the invasive trees can start changing soil conditions during early stages of establishment, more impacts found in the field study suggests that several soil properties, as well as the

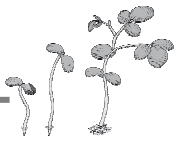
composition of soil bacteria communities and microbial activities need longer time since invasion to be altered.

Key words: exotic plants; nitrate; nitrogen; phosphomonoesterase; bacterial community; DGGE.

Introduction

Invasive plants are introduced alien species able to establish viable populations and to expand to areas distant from the sites of introduction (Richardson et al. 2000). Once established, invasive plants may affect native plant communities by reducing their diversity and abundance (Vilà et al. 2011). This effect may be caused directly by allelopathy and competition for resources (Callaway and Ridenour 2004; Vilà and Weiner 2004; Maron and Marler 2008) or indirectly by modifying the environment to the detriment of native species in their own benefit (Haubensak and Parker 2004; Niu et al. 2007). Recent reviews suggest that the overall effect of invasive plants is an increase of nutrient pools and acceleration of fluxes (Ehrenfeld 2003; Liao et al. 2008; Vilà et al. 2011; Castro-Díez et al. 2014). The alteration of ecosystem properties by plant invaders may also increase the habitat invasibility for other plants in a process named "the invasion meltdown" (Simberloff and Von Holle 1999; Von Holle et al. 2006). Moreover, the effects of invasive species may persist during years after the invader removal maintaining the risk of invasion and hampering the recovery of the ecosystem or restoration with native plants (Marchante et al. 2009; Von Holle et al. 2013).

Invasive plants may change the composition and activity of microbial communities. For instance, Hawkes et al. (2005) found that invasive grasses increased the abundance and change the composition of ammonium oxidizing bacteria, which caused greater nitrification rates in soil. Likewise, changes in soil pH caused by different plant species can alter the composition of the microbial community (Thoms and Gleixner 2013). Greater impacts on the composition and activity of soil microbial communities can be produced by invasive species differing from natives in nitrogen use strategies (Boudsocq et al. 2012), allelochemical compounds (Callaway et al. 2008; Lorenzo et al. 2013) or the quantity and chemical composition of plant tissues and root exudates (Wolfe and Klironomos 2005; Rodgers et al. 2008; Weidenhamer and Callaway 2010). Moreover, the establishment of positive plant-soil-microbe feedbacks in



the invaded range is considered as a cause of the invasion success of some species and a mechanism to alter microbial communities (De la Peña et al. 2010; Rodríguez-Echeverría et al. 2013). For instance, invasive species may accumulate soil pathogens, affecting native plants (e.g. the invasive weed, *Chromolaena odorata,* increased the abundance of the soil pathogenic fungi, *Fusarium semitectum*; Mangla et al. 2008)). Invasive species may also disrupt belowground mutualisms between native plants and arbuscular mycorrhizal fungi (Reinhart and Callaway 2006) or symbiotic nitrogen-fixing bacteria (Rodríguez-Echeverría 2010; Rodríguez-Echeverría et al. 2012). Lastly, plant invasion may also lead to the introduction of exotic soil mutualistic microorganisms (Rodríguez-Echeverría et al. 2011; Nuñez and Dickie 2014).

Riparian forest ecosystems are highly susceptible to plant invasion due to their more buffered temperatures and moister conditions compared with surrounding ecosystems (Hood and Naiman 2000). In addition, human activities, such as river canalization or flood regulation, cause the decline of native vegetation which forms gaps, giving exotic species a chance to be established (Zedler and Kercher 2004). In the Iberian Peninsula, the invasive trees *Ailanthus altissima* (Mill.) Swingle (Simaroubaceae) and *Robinia pseudoacacia* L. (Fabaceae) are found colonizing riparian ecosystems (Castro-Díez et al. 2009, 2012). *A. altissima* is native to China and North Vietnam while *R. pseudoacacia* is native to Appalachian Mountains (Southeast of USA) (Kowarik and Säumel 2007; Cierjacks et al. 2013). They are both included in the Spanish Atlas of Invasive Plants and considered among the 20 most harmful species in Spain and among the 100 worst invasive species in Europe (Sanz-Elorza et al. 2004; GEIB 2006; DAISIE 2009). Both species have allelopathic compounds in their tissues (Kowarik and Säumel 2007; Cierjacks et al. 2013) and they both have shown the ability to increase soil nitrate concentration and net nitrification rates in nutrient-poor soils likely due to high quality leaf litter of *A. altissima* and the ability of *R. pseudoacacia* to fix N_2 from the atmosphere (Rice et al. 2004; Gómez-Aparicio and Canham 2008b; Von Holle et al. 2013).

However, the effect of both species on the composition of soil microbial communities together with soil properties and nutrient mineralization rates in riparian ecosystems remains unexplored even when soil microorganisms control important ecosystem processes, such as mineralization of soil organic matter or soil nitrate production and assimilation (Booth et al. 2005; Myrold and Posavatz 2007).

The aim of this study was to assess the effects of the invasive trees, *A. altissima* and *R. pseudoacacia*, on soil properties and on the structure and activity of soil bacterial communities of riparian forest dominated by the native tree *Populus alba* L. (Salicaceae). We used two complementary approaches: 1) a field study comparing soil properties between invaded and paired non-invaded sites (*A. altissima*-*P. alba* and *R. pseudoacacia*-*P. alba*) and 2) a greenhouse experiment (GHE), where the invaders *A. altissima* and *R. pseudoacacia* and the native *P. alba* were grown for six months in a native soil. It is possible that there were pre-existing soil characteristics, which contributed to invasion of the tree species (Dassonville et al. 2008). Therefore, GHE growing exotic invasive trees in non-invaded soils allow to distinguish if differences observed in the field are due to the presence of the plant invader or to the preexisting site conditions (Ehrenfeld et al. 2001).

Materials and Methods

Field sampling

The study was conducted in the riparian zone of the Henares River (Tagus Basin, Central Spain), where *P. alba* is the dominant tree, which is accompanied by other native tree species, such as *Tamarix gallica* L., *Salix alba* L., *Populus nigra* L., *Fraxinus angustifolia* Vahl. and *Ulmus minor* Mill. (Martínez et al. 2000). We selected five sites (Table 1) where invaded patches by *A. altissima* or *R. pseudocacia* were close to native patches (i.e. vegetation dominated by *P. alba*). Two sites were invaded by the exotic *A. altissima* (Chiloeches and Guadalajara), two by *R. pseudoacacia* (El Encín and Jadraque), and one invaded

by both exotic trees (El Val). In this way we had three sites (replicates) per species.

Geographical coordinates, a soil taxonomic classification and vegetation cover of each study site are shown in Table 1. In each patch we selected five adult dominant trees (*P. alba*, *A. altissima* or *R. pseudoacacia*), which were considered as pseudo-replicates. Below the canopy of each tree, soil samples were collected in the seasons with greatest microbial activity, i.e. autumn (12-15 December 2011) and spring (17-25 April 2012). Each soil sample consisted of the mixture of four sub-samples taken at 1 m distance around the tree trunk by means of a metallic rectangular core (11 cm depth, 7.5 cm width). Soil samples were kept in polyethylene bags and carried to the lab, spread on trays, air-dried at room temperature, sieved (1 mm mesh) (Hawkes et al. 2005; Niu et al. 2007; Lorenzo et al. 2010) and divided in two parts. One was stored at -32 °C for bacterial DNA analyses and the other part was conserved at 4 °C for the analysis of soil properties (nutrients, percentage of organic matter, pH and mineralization rates). In the autumn, the litter layer (a 21 cm diameter surface) above each of the four soil subsamples was taken and pooled in a single sample. Litter samples were kept in paper bags and brought back to the lab, divided by plant part (leaf and woody), oven dried (60 ^{o}C ≥ 48 h) and weighed (Balance Sartorius BP211D, 0.0001 g) (Table 1).

In December 2011, we measured the basal perimeter of the selected trees (Table 1). In April 2012, we established a square plot (4 x 4 m) containing each sampling tree in the center. Within all square plots the canopy cover of the sampling tree species was greater than 75 %. In each square plot, we registered the herbaceous and shrubs species cover (%), as well as the cover of the sampling tree seedlings and saplings (Table 1).

Table 1. Geographical coordinates, altitude and soil classification of the study sites (CH = Chiloeches, G = Guadalajara, J = Jadraque, E = El Encín, V = El Val) and mean values (± SE, N = 5) of plant variables at understory level in the study patches: invaded (I) by *Ailanthus altissima* or *Robinia pseudoacacia* and dominated by the native *Populus alba* (N). Long. = Longitud; Lat. = Latitud; Alt. = Altitud; AA = *A. altissima*; RP = *R. pseudoacacia;* PA = *P. alba.*

Site name	Long.	Lat.	Alt. (m)	Soil classification (Dominant soil + associated soils)[a]	Patch[b]	Dominant tree species	Herbaceous cover (%)	Shrubs Cover (%)	Cover of tree seedlings/saplings (%)(DBH < 7 cm)[c]	Leaf litter (g)	Woody litter (g)	Total litter (g)
CH	3 ° 13´ W	40 ° 34´ N	609	Calcic Cambisol + Calcaric Regosol	I	*AA*	33 ± 11	13 ± 13	70 ± 17	58 ± 6	15 ± 2	73 ± 9
					N	*PA*	90 ± 4	13 ± 5	0 ± 0	25 ± 2	16 ± 3	41 ± 4
G	3 ° 11´ W	40 ° 37´ N	633	Calcic Cambisol + Calcaric Regosol	I	*AA*	44 ± 11	0 ± 0	66 ± 5	101 ± 8	133 ± 18	235 ± 19
					N	*PA*	75 ± 4	0 ± 0	38 ± 5	155 ± 18	61 ± 11	215 ± 21
J	2 ° 56´ W	40 ° 56´ N	800	Calcic Cambisol + Euthric Litosol + Rendzina + Chromic Luvisol	I	*RP*	92 ± 4	10 ± 5	26 ± 5	43 ± 4	30 ± 7	80 ± 10
					N	*PA*	98 ± 2	0 ± 0	0.2 ± 0.2	89 ± 23	38 ± 6	127 ± 23
E	3 ° 17´ W	40 ° 31´ N	594	Calcaric Fluvisol + Calcic Cambisol + Gleyo-calcaric Fluvisol	I	*RP*	48 ± 14	0 ± 0	34 ± 9	55 ± 4	31 ± 12	99 ± 11
					N	*PA*	3 ± 1	0 ± 0	38 ± 5	72 ± 8	24 ± 7	97 ± 2
V	3 ° 20´ W	40 ° 29´ N	587	Calcaric Fluvisol + Calcic Cambisol + Gleyo-calcaric Fluvisol	I	*AA*	86 ± 3	0 ± 0	24 ± 7	49 ± 10	47 ± 12	99 ± 18
					I	*RP*	92 ± 1	0 ± 0	0 ± 0	44 ± 12	46 ± 12	99 ± 24
					N	*PA*	29 ± 6	9 ± 2	25 ± 8	57 ± 5	22 ± 4	79 ± 7

[a] FAO/IIASA/ISRIC/ISSCAS/JRC, 2012. *Harmonized World Soil Database (version 1.2)*. FAO, Rome, Italy and IIASA, Laxenburg, Austria.
[b] I- Invaded, N- Non invaded
[c] Only for the dominant tree species

Greenhouse experiment

Soil from a non-invaded area dominated by *P. alba* in the Jadraque site (Table 1) was collected on 27 April 2012 to fill five 0.5 L plastic pots per species (*A. altissima*, *R. pseudoacacia* and *P. alba).* As a control, five additional pots were left without plants during the six-month experiment (named as "control t6"). In addition, an aliquot of the soil sample was taken at the beginning of the experiment (named as "control t0") (**Anexo, foto 3**). Seeds were collected in the field from at least 5 trees per species. Seeds were disinfected with 10 % bleach. *R. pseudoacacia* seeds were subsequently scarified mechanically with sand paper and wings of *A. altissima* seeds were removed. Seeds from *P. alba* were not manipulated before sowing. On 4 May 2012 we sowed the filled pots with seeds from the three target species to have one plant per pot. Sowed and control t6 pots were kept in an incubation chamber at 20 ºC with 12-12 h dark-light photoperiod. After 21 days, pots were moved to an experimental outdoors plot with 65 % of full sunlight (González-Muñoz et al. 2011) placed in the Botanical Garden of Alcalá de Henares (Madrid, Spain). Pots were irrigated when necessary and weeds were removed. Periodically the position of the pots was randomly changed to reduce the effect of micro-environment. After 182 days (six months), we harvested the above and belowground part of plants and took four soil subsamples with 1-cm diameter PVC tubes per pot, following the methodology of Ehrenfeld et al. (2001). Soil samples were air-dried and divided in two parts to perform bacterial DNA and soil properties analyses (see field study in the above section). The remaining soil in the pot was preserved at –32 ºC until it was washed in a sieve to separate the roots from the soil. Plants and clean roots were oven-dried at 60 ºC for at least 48 h and weighed (plant above and belowground biomass) (**Anexo, foto 4**). The above and belowground biomass ratio was calculated.

Soil properties

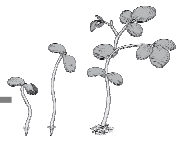
To analyze total nitrogen (N) and phosphorus (P) concentration, 0.5 g of soil were digested with H_2SO_4 and Cu-KSO_4. To analyze nitrate (NO_3^-–N) and ammonium (NH_4^+–N), 5 g of soil were mixed with 100 mL KCl 2 N and shaking the mix for 2 h. The solution was filtered using 0.45 µm Millipore filters and preserved at −20 ºC until analysis (Allen et al. 1986). The digested solution (total N and P) and the KCl solution (NH_4^+–N and NO_3^-–N) were analyzed with segmented flux autoanalyzer (Skalar San ++). The ratio between the concentration of NO_3^-–N and NH_4^+–N (NO_3^-–N:NH_4^+–N) was calculated. Total mineral N was calculated as the sum of NO_3^-–N and NH_4^+–N. The percentage of total N belonging to organic N was calculated as:

$$\text{Organic N (\%)} = \frac{\text{total soil N} - \text{total mineral N}}{\text{total soil N}} \times 100$$

The organic matter (OM) of the soil was determined by weighing the dry soil (at 105 °C, ≥ 48 h) before and after ignition at 400 ºC for 24 h (Nelson and Sommers 1973). To measure the soil pH, 20 g of soil were mixed with 40 mL of distilled water (slurry texture) (Allen et al. 1974). The mixture was measured for pH using a pH-meter (micropH 2001, Crison Instruments, Barcelona, Spain).

Mineralization rates

The potential net nitrification, ammonification and mineralization rate of soil N were assessed as the difference in NO_3^-–N, NH_4^+–N, and the sum of both, respectively, in the soil before and after incubation at 30 ºC for 14 days. Two aliquots of five grams per soil sample (both from the field and the experimental pots) were taken and mixed up with 15 g of washed sand (SiO_2, Panreac) in a 200 mL polypropylene bottle. In one of the soil aliquots, mineral N was extracted immediately while in the other aliquot mineral N was extracted with 6 mL of distilled water after incubation, at 30 ºC for 14 days. Mineral N was extracted with 100 mL KCl 2M during 2 h in a shaker. The solution was then filtered through 0.45 µm Millipore filters and preserved at -20 ºC until analysis



(Allen et al. 1986). NH_4^+–N and NO_3^-–N were analyzed with segmented flux autoanalyzer (Skalar San ++).

The potential mineralization rate of soil P was estimated by the activity of the acid phosphomonoesterase (PME) enzyme, which is the main enzyme responsible for organic P mineralization (Olander and Vitousek 2000). This activity was determined as the liberation of p-Nitrophenol (*p*-NP) from p-Nitrophenyl phosphate (*p*-NPP), which is an organic phosphate analog. Five grams of each sample (both from the field and the GHE) were mixed with 0.2 mL of 0.1 M of maleate buffer (pH = 6.5) and 0.5 mL of *p*-NPP substrate. The mixture was incubated at 30 °C for 90 min. The reaction was stopped with cold temperature (4 °C, 15 min) and then 2 mL of NaOH and 0.5 mL of $CaCl_2$ 0.5 M were added until alkaline pH (pH ≈ 9) where *p*-NP turns yellow. The mixture was centrifuged at 3000 rpm for 15 min. The *p*-NP concentration was measured with a spectrophotometer at 398 nm. The PME activity was expressed as the number of µmol of *p*-NP produced per grams of dry soil per hour.

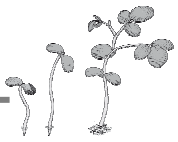
DNA extraction and amplification and Denaturing Gradient Gel Electrophoresis analysis (DGGE)

DNA was extracted from each soil sample leading to total of 110 extractions (5 trees x 11 patches x 2 seasons) from the field and to 20 extractions ((5 x 3 trees) + 5 controls) from the GHE. Soil DNA was extracted from 0.25 g of soil per sample using a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., CA). Specific eubacteria primers (primer 1, 5'-C CTACGGGAGGCAGCAG-3'; primer 2, 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGG GAGGCAGCAG-3') were used to amplify 16S rRNA genes (Muyzer et al. 1993) from total DNA extracted. All reactions were carried out in a final volume of 25 µL containing 2.5 µL of buffer (160 mM $(NH_4^+)_2SO_4$, 670 mM Tris–HCl, pH 8.8, 0.1 % Tween-20, 25 mM $MgCl_2$) (BIORON, Germany), 400 nM of each primer, 200 µM dNTPs, 0.5 U of DFS-Taq polymerase (BIORON, Germany), and 1 mL

of template DNA. The PCR conditions were: an initial denaturing step at 94 °C for 5min followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C and 30 s at 72 °C, followed by a final extension step at 72 °C for 30 min. The size and integrity of PCR fragments were checked in agarose gel electrophoresis (1 %, w/v) stained with GelRed™. All PCRs were performed using a GeneAmp 9700 (Applied Biosystems, PerkinElmer, CA, USA). For field-collected samples, equal volumes of each PCR were mixed to get a single sample per season, site and tree species. In this way we mixed variability of pseudo-replicates but maintained three replicated sites per species. Each mixture was analyzed by Denaturing Gradient Gel Electrophoresis (DGGE). For GHE samples, individual PCR products were run using DGGE. We performed DGGE with a DGGE-2001 system from CBS Scientific (CA, USA). 15 µL of each PCR product (or mix of PCR products in the case of the field samples) was used for DGGE analysis. Gels contained 8 % (w/v) acrylamide and a linear gradient of 45 to 68 % denaturant were used. The 100 % denaturing acrylamide was defined as containing 7 M urea and 40 % (v/v) formamide. Gels (22 cm × 17 cm) were run in 21 L 1× TAE buffer at 20 V for 15 min, followed by 16 h at 70 V and maintained at a constant temperature of 60 °C. Gels were stained for 20 min in 1X GelStarR® and destained for 30 min in distilled water prior to visualization. Gel Compar II (Applied Maths, Belgium) was used to obtain the DGGE gel bands on digitalized images of the gels.

Statistical analysis

For each invaded-native situation (*A. altissima-P. alba*, *R. pseudoacacia-P. alba*), soil samples collected in the field below native and invasive trees were ordered according to their values of soil variables (soil properties and mineralization rates) using redundancy analysis (RDA), which is a linear constrained form of the principal component analysis (PCA) (Lepš and Šmilauer 2003). We used species, season, total litter and tree basal perimeter as potential predictors. Permutations for the Monte Carlo test (9999) were conducted and restricted according to three independent sites replicated across



two seasons (spring and autumn). Site was included as a covariate to account for its variability. Temporal dependency in data due to repeated measures was accounted for in the restriction of permutations at the whole-site level. In support to the direct ordination analysis we constructed a *t*-value biplot for species which allowed us to test and plot the relationship between the predictor variable (species) and each soil variable in a multivariate plot. This analysis assumes that the relationship between the predictor variable and each soil variable is significant ($P < 0.05$) if t values of respective regression coefficients are > 2 units. Significant relationships between soil variables and predictors are indicated with circles to distinguish positive from negative relationships (Van Dobben circles; Lepš and Šmilauer 2003). Vectors (soil variables) fully falling within a Van Dobben circle indicate that they are significantly different between two levels of the predictor variable (species) (Lepš and Šmilauer 2003). Similarly, greenhouse soil samples were ordered according to their values of soil variables using RDA, with species as potential predictor. *T*-value biplots were also constructed to compare soil properties of control t6 samples with soil samples conditioned by the different tree species (*A. altissima*, *R. pseudoacacia* and *P. alba*). In addition, *t*-value biplots were constructed to compare soil properties between samples conditioned by the growth of invasive species and those conditioned by the growth of the native *P. alba*. Plant biomass was compared across species using one-way ANOVA.

Bacterial richness was calculated as the total number of DGGE gel bands per sample. Bacterial diversity was estimated from the total number of bands and their relative intensity following Lorenzo et al. (2010). Gel bands were classified according to their intensity in four categories. Diversity was calculated using a modification of the Shannon index,

$$H' = -\Sigma [(n_i/N)\mathrm{Ln}\ (n_i/N)]$$

where n_i had one of four possible values (1–4) depending on band intensity and N the total number of bands (Lorenzo et al. 2010). Statistical differences in soil

bacteria richness and diversity of soils from field-collected samples were assessed using two-way ANOVA with species and season as fixed factors. To assess differences in diversity and richness of bacterial communities of soils from the experiment, one way ANOVA with species as fixed factor was used. Differences in soil bacteria community based on the DGGE results were assessed by Non-metric Multidimensional Scaling (NMDS) analysis, one of the most effective ordination methods for ecological community data (McCune and Grace 2002). The sample positions on the NMDS biplot were calculated using Sorensen distances from the original data. Pair-wise correlations between NMDS axis and soil variables were performed in order to know the likely effect of the different soil variables on the structure of bacterial communities. To perform the pair-wise correlations with NMDS axis, we used the mean of 5 trees per site and tree species of each soil variable in the field study (n = 3) and individual replicates of each soil variable in the GHE (n = 5). RDA and *t* value biplots were conducted in CANOCO 4.5 (Lepš and Šmilauer 2003). ANOVA was conducted using R package 3.0.2 (R Development Core Team 2013). NMDS were performed using Community Analysis Package (CAP) 2004, V. 3.1 (www.piscies-conservation.com). Pair-wise correlations were performed in JMP, Version 7 (SAS Institute Inc., Cary, NC 1989-2007).

Results

Soil properties and mineralization rates

In the field study, soil from patches invaded by *A. altissima* had higher NO_3^-–N and NO_3^-–N:NH_4^+–N ratio and lower total N, OM, NH_4^+–N, PME activity, potential rates of net nitrification and total N mineralization than soils collected in adjacent native *P. alba* patches (*t*-value biplot, $P < 0.05$) (Fig. 1 a). The lower soil total N was attributed to lower organic N in *A. altissima* (98.14 ± 0.17 %) than in *P. alba* (98.88 ± 0.09 %) field soils (Table S1). Patches invaded by *R. pseudoacacia* had higher soil NO_3^-–N, NO_3^-–N:NH_4^+–N ratio, total N, total mineral N, potential net ammonification rate and PME activity (*t*-value biplot, $P < 0.05$) than adjacent *P. alba* patches (Fig. 1 b). In this case, the higher soil total N was attributed to higher mineral N in *R. pseudoacacia* than in *P. alba* field soils (Fig. 1 b).

In the GHE, after six months of growth in a native soil, *P. alba* showed the greatest mean plant biomass, although differences were marginally significant (Table 2). The three species differed in the allocation between above and belowground biomass, the ratio being the largest in *R. pseudoacacia* and the smallest in *A. altissima*. *P. alba* showed an intermediate value which did not differ from either of the exotic trees (Table 2).

Table 2. Values (mean ± SE, n = 5) of total, aboveground and belowground plant biomass and the ratio between above and belowground biomass (A:B ratio) for each plant species (*Ailanthus altissima, Robinia pseudoacacia* and *Populus alba*) after six months of growth in the greenhouse experiment.

Variables	***A. altissima***	***R. pseudoacacia***	***P. alba***
Plant aboveground biomass (g)	0.61 ± 0.12 b	0.89 ± 0.15 ab	1.15 ± 0.08 a
Plant belowground biomass (g)	1.26 ± 0.13 ab	0.87 ± 0.13 b	1.34 ± 0.11 a
Total plant biomass (g)	1.87 ± 0.19 a	1.76 ± 0.25 a	2.48 ± 0.16 a
A:B ratio	0.49 ± 0.11 b	1.07 ± 0.19 a	0.87 ± 0.07 ab

Different letters in a row mean significant differences between species (ANOVA, Tukey HSD test, $P < 0.05$)

Soil properties

(**1**) Total nitrogen (%)
(**2**) Organic matter (%)
(**3**) Nitrate (mg kg^{-1})
(**4**) Ammonium (mg kg^{-1})
(**5**) NO_3^--N:NH_4^+-N ratio
(**6**) Total mineral N (mg kg^{-1})
(**7**) pH
(**8**) Total phosphorus (%)
(**9**) Net nitrification rate (µg g $soil^{-1}$ day^{-1})
(**10**) Net ammonification rate (µg g $soil^{-1}$ day^{-1})
(**11**) Net N mineralization rate (µg g $soil^{-1}$ day^{-1})
(**12**) PME activity (µmol *p*-NP g $soil^{-1}$ h^{-1})

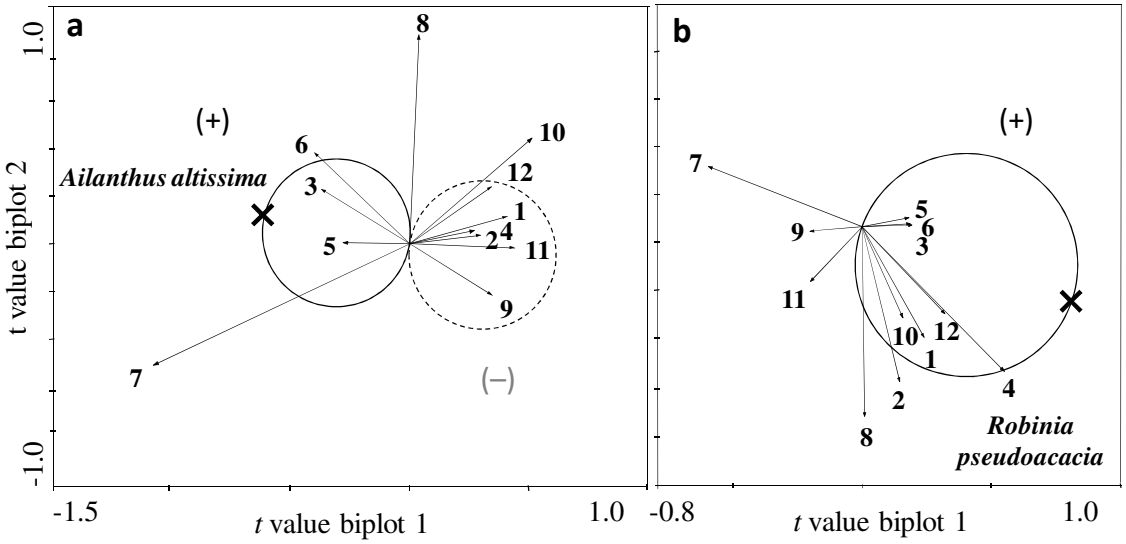


Figure 1. Field sampling: *t*-value biplots for the relationship between soil properties (vectors with numbers) and the invasion of *Ailanthus altissima* (a) and *Robinia pseudoacacia* (b) compared with non-invaded patches (dominated by *Populus alba*). Crosses indicate the centroids of the invasive trees, *A. altissima* and *R. pseudoacacia*. Soil property vectors fully falling within a Van-Dobben circle (Lepš and Šmilauer 2003) indicate a significant relationship between the focal soil property and the presence of the invasive species: *A. altissima* (a) and *R. pseudoacacia* (b). Relationships in *t* value biplots may be positive (solid line circle) or negative (dashed line circle) and are based on *t* values of regression coefficients of soil properties expressed as linear combinations of *A. altissima* or *R. pseudoacacia* presence. Mean (± SE) values of soil variables used are available in supplementary material (Table S1).

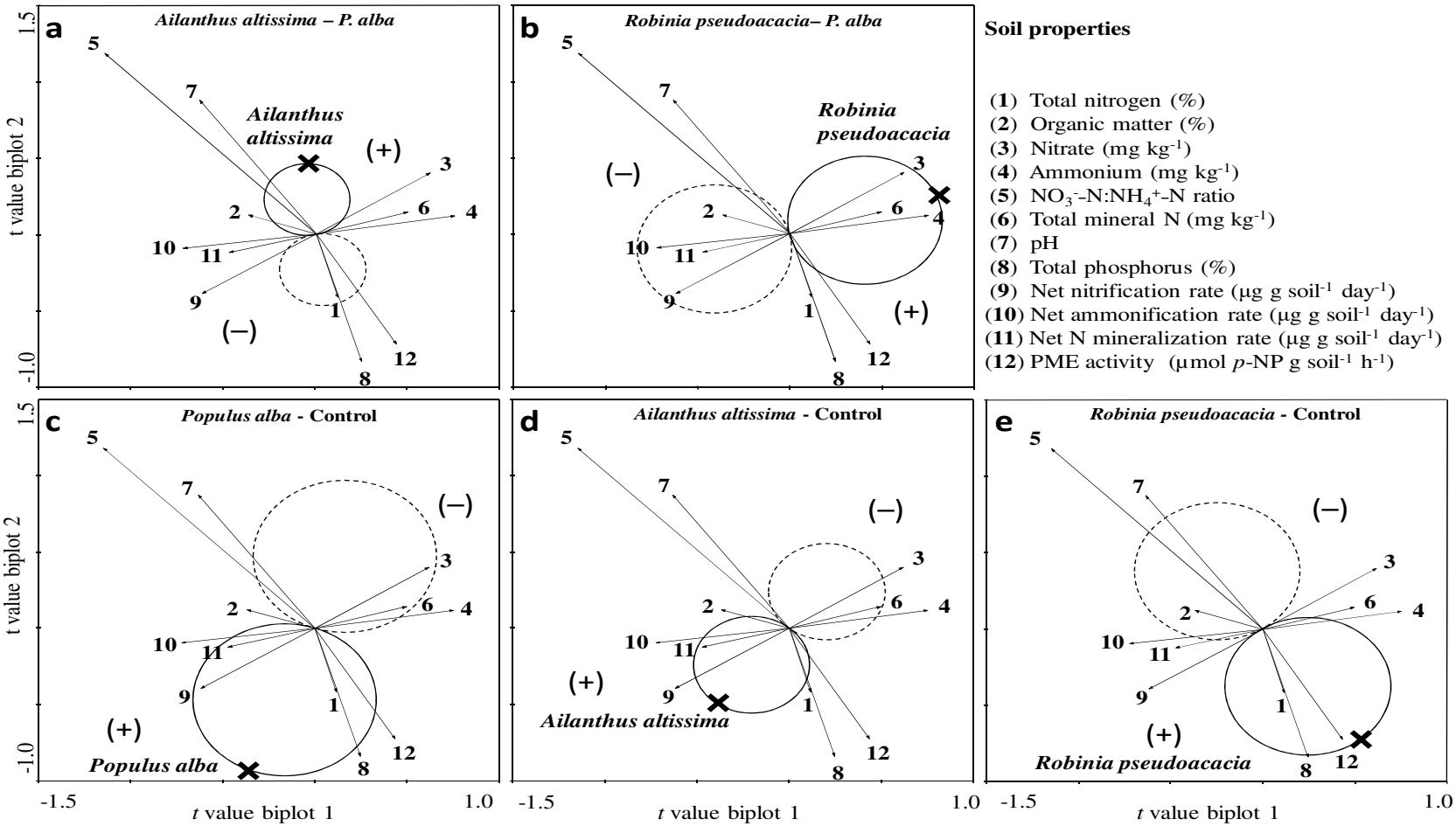


Figure 2. Greenhouse experiment: *t*-value biplots comparing soil properties (vectors with numbers) in samples conditioning by the growth during six months of *P. alba* samples with those of *Ailanthus altissima* (a) and those of *Robinia pseudoacacia* (b). *T*-value biplots comparing soil properties of control samples (soils where no plant grew) with soil samples conditioning by the growth during six months of the tree species: *Populus alba* (c), *Ailanthus altissima* (d), *Robinia pseudoacacia* (e). Crosses indicate the centroids of the tree species (*A. altissima*, *R. pseudoacacia,* or *P. alba)*. Vectors fully falling within a Van-Dobben circle (Lepš and Šmilauer 2003) indicate a significant relationship between the focal soil property and the tree species. Relationships in *t*-value biplots may be positive (solid line circle) or negative (dashed line circle) and are based on *t* values of regression coefficients of soil properties expressed as linear combinations of *A. altissima*, *R. pseudoacacia, P. alba* or control samples. Mean (± SE) values of soil variables used are available in supplementary material (Table S2).

Soil bacterial community

The NMDSs performed with the data obtained from DGGE of field soil samples had stress values lower than 0.20 and therefore allowed reliable interpretations (McCune and Grace 2002). The composition of field soil bacterial communities differed between invaded and non-invaded patches (Fig. 3). Both in autumn and spring, *A. altissima* and *P. alba* soil bacterial communities were separated by axis 2, while sites were separated along axis 1 (Fig. 3 a and b). Axis 2 of the NMDS for autumn was negatively correlated with soil NO_3^-–N concentration (r^2 = -0.91, P = 0.013) and NO_3^-–N:NH_4^+–N ratio (r^2 = - 0.94, P = 0.006), and positively correlated with potential net nitrification rate (r^2 = 0.93, P = 0.008) (Fig. 3 a). These correlations indicated that in autumn, the soil bacterial community in *A. altissima* patches was related with greater NO_3^-–N concentration and NO_3^-–N:NH_4^+–N ratio and with lower net nitrification rate (i.e. greater NO_3^-–N assimilation). However, in spring we found a different interaction since axis 1 of the NMDS was positively correlated with potential net ammonification rate (r^2 = 0.95, P = 0.004) and PME activity (r^2 = 0.87, P = 0.023) (Fig. 3 b), indicating that the bacterial community of Guadalajara was related to greater net ammonification rates and PME activity, as compared to Chiloeches and El Val sites. In the case of *R. pseudoacacia - P. alba* sites, axis 1 of the NMDS plot mostly separated sites, but differences between invaded and non-invaded plots within sites were more subtle (Fig. 3 c and d). The largest difference between invaded and native soils was found in El Encín in spring (Fig. 3 c). The axis 2 of the NMDS for spring soil samples was positively correlated with soil NH_4^+–N concentration (r^2 = 0.88, P = 0.020) and PME activity (r^2 = 0.93, P = 0.008) (Fig. 3 d).

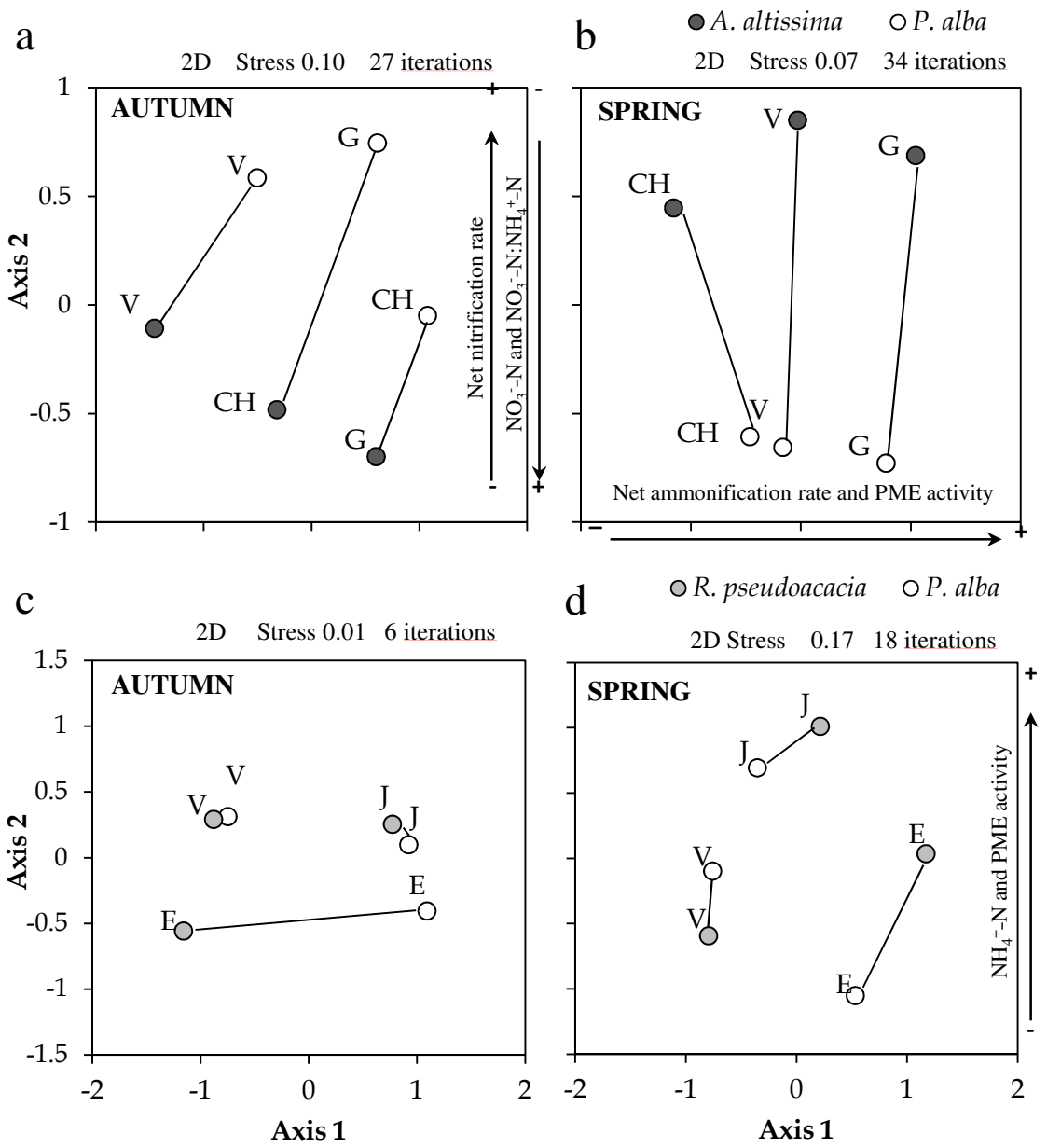


Figure 3. Field sampling: Two dimensional Non Metric Multidimensional Scaling (NMDS) plots, based on DGGE data, showing soil samples clustered according to the composition of soil bacteria community in *Ailanthus altissima* - *Populus alba* (a and b) and *Robinia pseudoacacia* - *Populus alba* (c and d) paired sites, in autumn (a and c) and spring (b and d). CH = Chiloeches site; G = Guadalajara site; V = El Val site; J = Jadraque site; E = El Encín site. White points mean native patches and grey points invaded patches. Lines join invaded and non-invaded patches of each site to highlight the potential effect of the invader. Arrows beside the axis indicate significant ($P < 0.05$) pair wise correlations between the axes and the indicated soil variables: ammonium concentration (NH_4^+-N), nitrate concentration (NO_3^--N), nitrate:ammonium ratio (NO_3^--N:NH_4^+-N), phosphomonoesterase activity (PME), net ammonification and nitrification rates.

In the GHE, NMDS plot did not show clear differences in bacterial community composition among plant species (Fig. 4). Axis 1 was positively correlated with NH_4^+–N (r = 0.55, *P* = 0.010) and total mineral N (*r* = 0.45, *P* = 0.039) concentrations and negatively with net ammonification rate (*r* = -0.50, *P* = 0.020). The remaining soil variables did not significantly correlate with NMDS axes (*P* > 0.05). In the field, species richness and diversity of soil bacteria did not differ between invaded (either by *A. altissima* or *R. pseudoacacia*) and non-invaded patches but both variables were greater in spring than in autumn (Fig. S1). Similarly, in the GHE there were not significant differences in bacterial richness or diversity between soils where the different species grew (Fig. S2).

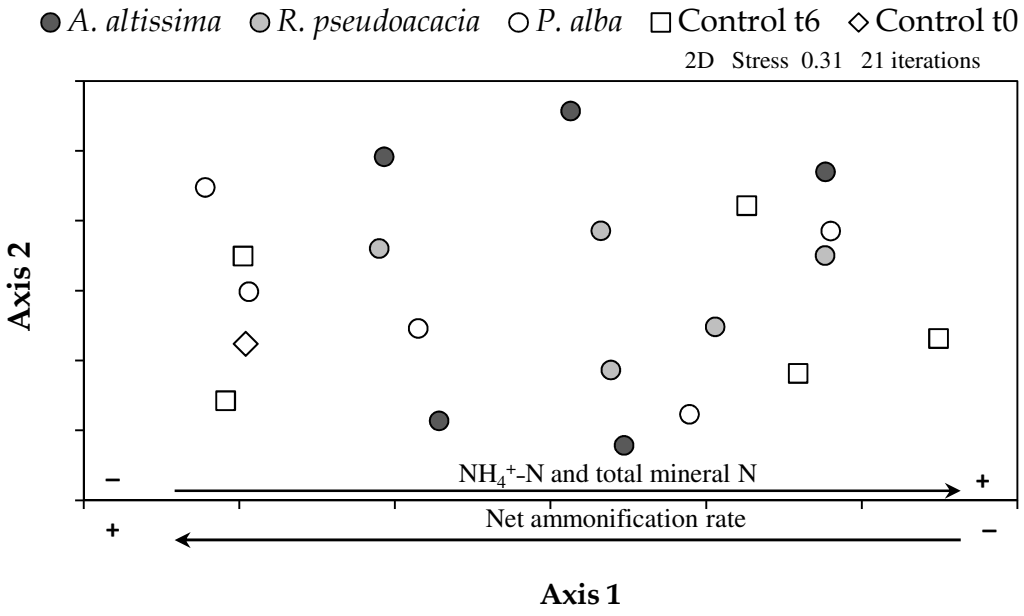


Figure 4. Greenhouse experiment: Two dimensional Non Metric Multidimensional Scaling (NMDS) plot showing soil samples clustered according to their composition of soil bacteria community under the influence of the growth of *Ailanthus altissima*, *Robinia pseudoacacia* and *Populus alba* during 6-months and without the influence of any plant growth at the beginning (Control t0) and the end (Control t6) of the 6-months experiment. Arrows beside the axis indicate significant (*P* < 0.05) pair-wise correlations between the axis and the indicated soil variables: ammonium concentration (NH_4^+-N), total mineral N and net ammonification rate.

Discussion

Both the field and the GHE results consistently indicated the capability of invasive species to alter some soil properties. *R. pseudoacacia* increased mineral N (NO_3^-–N and NH_4^+–N) of riparian forest dominated by *P. alba*. This resulted from the N-fixing ability of *R. pseudoacacia*, which can directly release N by means of root exudates and increase soil fertility (Zahran 1999; Fustec et al. 2010). A similar trend was previously reported by other studies comparing soils under *R. pseudoacacia* and under pine or oak trees (Rice et al. 2004; Von Holle et al. 2013). By contrast, *A. altissima,* decreased total soil N both in the field and in the GHE, mainly due to a decrease of the organic N, which represented more than 95 % of total N. In a previous study we found that *A. altissima* leaf litter decomposed faster than *P. alba* leaf litter, probably due to the higher litter quality of the former (Medina-Villar et al. 2015). The low organic N in *A. altissima* patches suggests that the rate of soil organic N loss by mineralization was higher than the rate of organic N gain by litter decomposition. However, in the GHE, six-month plants produced a negligible amount of leaf litter which unlikely could affect soil N. Consequently, belowground mechanisms may also account for the lower total N in *A. altissima* than in *P. alba* soils. These mechanisms may include that *A. altissima* 1) had lower production of N-rich root exudates or root litter, 2) had higher uptake of N-rich organic monomers (e.g. amino acids, nucleic acids), 3) enhanced the activity of N-mineralizing microorganisms by improving the physical connection between organic N and soil decomposers (Schimel and Bennett 2004). The suggested higher N mineralization rate in *A. altissima* soils contrasts with the lower net N mineralization found in the field experiment. However, this apparent contradiction is compatible with an increase of gross N mineralization in *A. altissima* soils, but a faster loss of mineral N either by a faster increase of the uptake by microorganisms or by a faster increased of denitrification rates, promoted by the higher nitrate availability in *A. altissima* soils (Moreau et al. 2015).

The rest of the results with significant difference between invasive and native trees found in the field were not found in the GHE, and vice versa, possibly because the effects of these species on soil properties may vary with invasion time or plant age (Strayer et al. 2006). The lower soil OM in *R. pseudoacacia* than in *P. alba* soils, which was only found in the GHE, may be due to the lower root biomass found for the invader seedlings, given that dead roots are an important source of OM for the soil (Frank and Groffman 2009). In addition, N mineralization activity has been found to be affected by plant age (Tolman et al. 1990; Abbès et al. 1995; Côté et al. 2000), which may explain that *R. pseudoacacia* was found to increase net ammonification in the field, but to decrease it in the GHE, along with net nitrification. The higher PME activity in *R. pseudoacacia* field soils as compared with *P.alba* ones, is in accordance with the stimulation of this activity reported for legumes, which demand P for N-fixation (Reinsvold and Pope 1987; Makoi and Ndakidemi 2008). However, the fact that this trend was not found in the GHE may be explained because PME activity increases with plant age and size (Makoi and Ndakidemi 2008; Orczewska et al. 2012). Low root development of *R. pseudoacacia* plants in the GHE also implied low root surface to exude PME enzymes and to support the PME enzymes associated to root cells (Rejsek et al. 2012).

Changes in bacteria-related processes, such as nutrient cycling, might be explained by modifications of soil bacterial communities (Hawkes et al. 2005). Accordingly, in our study, the change of soil bacterial community in *A. altissima* patches, compared to that in *P. alba* patches, was related to a lower potential net nitrification rate and with higher NO_3^-–N and nitrate:ammonia ratio. Therefore, *A. altissima* may be affecting soil bacteria related to net nitrification rates (e. g. nitrifiers, nitrate-reducing bacteria or any microorganisms that can take NO_3^-–N). Due to the limitation of the DGGE technique (Fakruddin et al. 2013), we could not determine which organisms changed in the studied bacterial communities. However, our study constitutes a first step to assess differences in bacterial communities between invaded and non-invaded riparian forests. In spite of its limitations, DGGE is still a valid and useful methodology to describe

soil communities and explore differences among them (Souza-Alonso et al. 2015). Recent studies have also demonstrated that DGGE and new generation sequencing techniques, such as pyrosequencing, are equally useful to detect differences in soil microbial communities (Buscardo et al. 2015). Differences in soil bacterial communities between *R. pseudoacacia* and *P. alba* patches were only appreciable at one site and season (El Encín in spring) and were related to greater ammonium concentrations and PME activity in *R. pseudoacacia* than in *P. alba* soils. Other studies soils from maize crops also found that changes in PME activity coupled with changes in soil bacterial community (Kandeler et al. 2002). Besides, dominant tree species affected the microbial communities more in spring than in autumn likely due to the higher tree activity and advanced decomposition of the leaf and root litter (Thoms and Gleixner 2013). GHE also showed that the composition of soil bacterial communities was related to N cycle (i.e. ammonium concentration, net ammonification rate and total mineral N concentration). However, the lack of differences among tree species in soil bacterial communities indicated that more than six months were needed for tree species to modify soil bacterial communities.

The increase in overall N availability due to *R. pseudoacacia* invasion may hinder restoration with native species (Haubensak and Parker 2004; Niu et al. 2007), although some native species could be suitable for the restoration of N-enriched invaded soils (Rodríguez-Echeverría et al. 2015). Greater nitrate in invaded than in non-invaded soils can persist up to 14 years after the removal of the invasive species (Von Holle et al. 2013). Elevated nitrate in invaded patches also increases the probability of nitrate to be leached and reach groundwater and freshwater ecosystems (Cameron et al. 2013). Nitrate accumulation in water stream has also been reported in wetlands adjacent to *R. pseudoacacia* stands (Williard et al. 2005). Future studies on the suitability of soils conditioned by *A. altissima* or *R. pseudoacacia* for the growth of native species, and their usefulness to reduce N leaching, are needed to assess the consequences of soil modification by these invasive species on restoration.

Conclusions

Our study showed the capability of *A. altissima* and *R. pseudoacacia* to alter soil properties of riparian forests dominated by *P. alba*. These alterations were species-specific with *A. altissima* decreasing total N and *R. pseudoacacia* increasing mineral N. Soil bacterial communities in the field differed between invaded and non-invaded soils, these differences were greater between *A. altissima* and *P. alba* than between *R. pseudoacacia* and *P. alba*. These differences were related to nitrate concentration and net nitrification rates in *A. altissima* soils and to ammonium concentration and PME activity in *R. pseudoacacia* soils. Our study also showed that the studied invasive species can alter some soil properties, such as N concentrations, in just six months but the composition of soil bacterial community and soil microbial activity needs longer time of invasion to be affected.

Acknowledgements

This study was supported by the projects CGL2011-16388/BOS of the *Ministerio de Economía y Competitividad* of Spain and POII10-0179-4700 of the *Junta de Comunidades de Castilla-La Mancha*. Silvia Medina Villar was supported by a grant from the *Ministerio de Economía y Competitividad* of Spain (FPI fellowship, BES-2011-048379). Susana Rodríguez-Echeverría has an Investigador IF Development Grant (IF/00462/2013) from the FCT and the European Social Fund. Paula Lorenzo is supported by a post-doctoral grant (SFRH/BPD/88504/2012) from the FCT and the European Social Fund. We are grateful to the support of the REMEDINAL3-CM MAE-2719 network (Comunidad de Madrid). We acknowledge to Dr. Raúl Ochoa-Hueso for the help in the PME analysis, to Guillermo Valle Torres for the field and greenhouse work collaboration and to Dr. Jaime Madrigal-González for the help in statistical analyses.

Supplementary Information

Table S1. Field sampling: Mean (± SE, n = 60) total nitrogen (N), soil organic matter (OM), nitrate (NO_3^--N), ammonium (NH_4^+-N), NO_3^--N:NH_4^+-N ratio, total mineral N, pH, total phosphorus (P), net nitrification rate, net ammonification rate, net N mineralization rate, phosphomonoesterase (PME) activity and organic N in patches invaded by *Ailanthus altissima* or by *Robinia pseudoacacia* and in adjacent patches dominated by the native *Populus alba*.

	***A. altissima*-*P. alba* pathes**		***R. pseudoacacia*-*P. alba* patches**	
Soil properties	*A. altissima*	*P. alba*	*R. pseudoacacia*	*P. alba*
Total N (%)	0.22 ± 0.01	0.26 ± 0.01	0.28 ± 0.01	0.23 ± 0.01
OM (%)	4.2 ± 0.2	5.3 ± 0.3	5.2 ± 0.2	4.6 ± 0.2
NO_3^--N (mg kg^{-1})	34.5 ± 4.9	15.6 ± 2.2	53.3 ± 5.6	18.3 ± 2.5
NH_4^+-N (mg kg^{-1})	8.3 ± 0.7	12.7 ± 0.8	11.9 ± 0.5	10.0 ± 0.5
NO_3^--N : NH_4^+-N ratio	4.5 ± 0.5	1.5 ± 0.3	4.5 ± 0.4	1.9 ± 0.3
Total mineral N (mg kg^{-1})	42.8 ± 5.4	28.3 ± 2.3	65.2 ± 5.8	28.3 ± 2.6
pH	8.19 ± 0.03	8.17 ± 0.03	7.98 ± 0.04	8.15 ± 0.04
Total P (%)	0.059 ± 0.004	0.055 ± 0.003	0.056 ± 0.003	0.053 ± 0.003
Net nitrification rate (µg g^{-1} day^{-1})	-2.5 ± 0.3	-1.0 ± 0.1	-3.7 ± 0.4	-1.3 ± 0.2
Net ammonification rate (µg g^{-1} day^{-1})	6.0 ± 0.3	6.4 ± 0.3	7.8 ± 0.4	5.9 ± 0.3
Net N mineralization rate (µg g^{-1} day^{-1})	3.5 ± 0.4	5.3 ± 0.4	4.1 ± 0.6	4.7 ± 0.3
PME activity (µmol p-NP g $soil^{-1}$ h^{-1})	1.4 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.2 ± 0.1
Organic N (%)	98.1 ± 0.2	98.9 ± 0.1	97.7 ± 0.2	98.7 ± 0.1

Table S2. Greenhouse experiment: Mean (± SE, n = 5) soil organic matter (OM), total nitrogen (N), total phosphorus (P), nitrate (NO_3^--N), ammonium (NH_4^+-N), NO_3^--N:NH_4^+-N ratio, total N mineral, net nitrification rate, net ammonification rate, net N mineralization rate, phosphomonoesterase (PME) activity and pH of soils where the species, *Ailanthus altissima*, *Robinia pseudoacacia* and *Populus alba* grew during six months and soils without plants at the beginning (Control t0) and after the six months (Control t6) of the experiment.

		Mean (SE)			
Soil properties	**Control t0***	**Control t6**	***A. altissima***	***R. pseudoacacia***	***P. alba***
Total N (%)	0.164	0.162 ± 0.004	0.168 ± 0.004	0.183 ± 0.003	0.186 ± 0.006
OM (%)	3.17	4.07 ± 0.10	4.14 ± 0.09	3.57 ± 0.11	4.09 ± 0.06
NO_3^--N (mg kg^{-1})	38.4	18.5 ± 3.2	13.6 ± 1.9	19.3 ± 1.9	10.1 ± 3.4
NH_4^+-N (mg kg^{-1})	25.9	33.1 ± 9.2	17.1 ± 1.6	39.6 ± 6.9	22.3 ± 5.8
NO_3^--N:NH_4^+-N ratio	1.48	0.77 ± 0.22	0.82 ± 0.11	0.54 ± 0.11	0.63 ± 0.22
Total mineral N (mg kg^{-1})	64.2	51.5 ± 8.9	30.6 ± 2.6	58.8 ± 7.0	32.5 ± 4.4
pH	8.020	7.98 ± 0.06	7.95 ± 0.05	7.78 ± 0.04	7.85 ± 0.14
Total P (%)	0.036	0.037 ± 0.001	0.038 ± 0.001	0.040 ± 0.001	0.040 ± 0.001
Net nitrification rate (μg g^{-1} day^{-1})	-2.72	-1.29 ± 0.22	-0.95 ± 0.13	-1.36 ± 0.14	-0.71 ± 0.25
Net ammonification rate (μg g^{-1} day^{-1})	12.9	12.4 ± 0.7	13.6 ± 0.1	11.9 ± 0.5	13.2 ± 0.3
Net N mineralization rate (μg g^{-1} day^{-1})	10.15	11.1 ± 0.6	12.6 ± 0.1	10.5 ± 0.5	12.5 ± 0.3
PME activity (μmol p-NP g $soil^{-1}$ h^{-1})	0.81	0.74 ± 0.04	0.94 ± 0.08	1.1 ± 0.1	0.90 ± 0.04
Organic N (%)	96.1	96.8 ± 0.6	98.2 ± 0.2	96.8 ± 0.4	98.2 ± 0.3

*No replication for these samples.

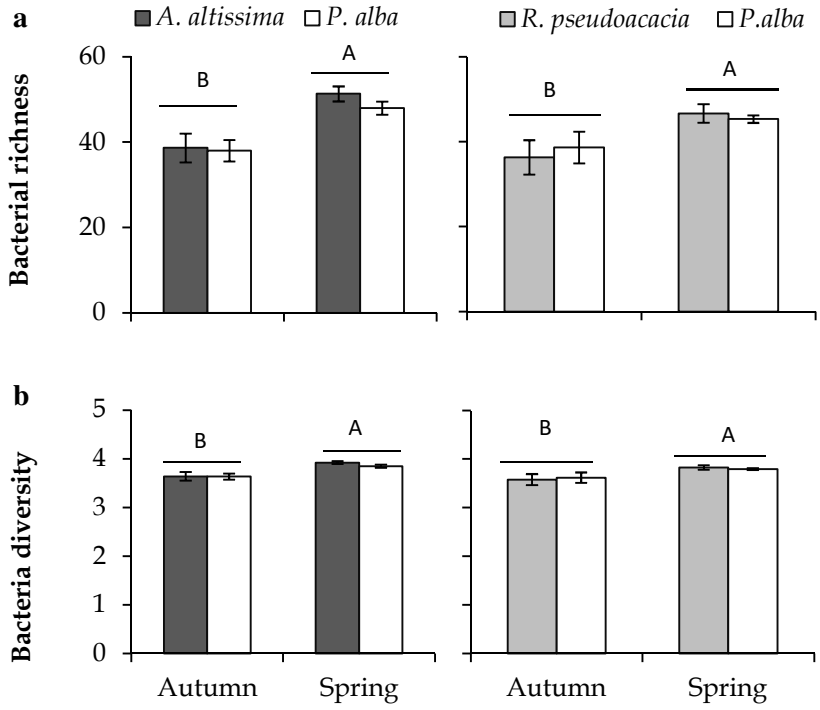


Figure S1. Mean (± SE, n = 3) bacterial richness (a) and bacterial diversity (b) of soils collected in the field under *Ailanthus altissima*, *Robinia pseudoacacia* and *Populus alba* trees in autumn (2011) and spring (2012). Different capital letters mean significant differences between seasons (ANOVA, $P < 0.05$). No significant differences were found among plant species.

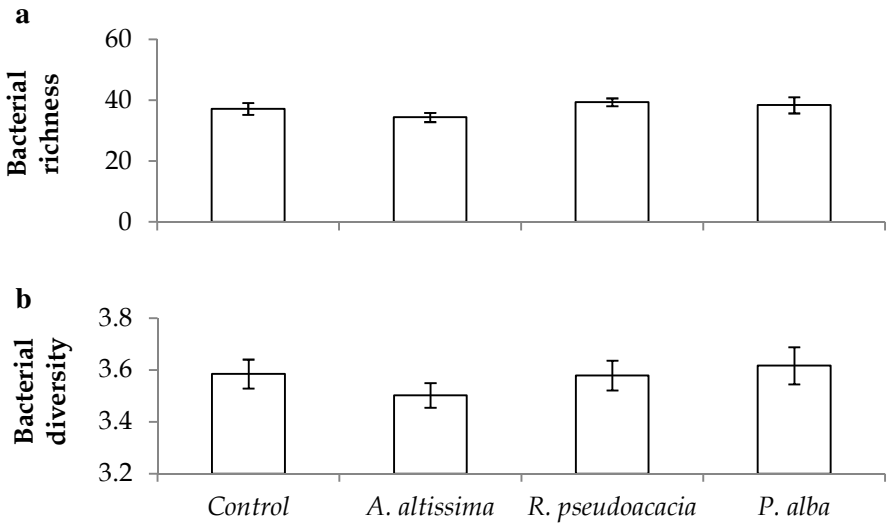


Figure S2. Mean (± SE, n = 5) bacterial richness (a) and bacterial diversity (b) of soils where *Ailanthus altissima*, *Robinia pseudoacacia* and *Populus alba* grew during six months and control soils (with no plant grew). No significant differences in bacterial richness and diversity were found among different soils (ANOVA, $P > 0.05$).

Capítulo 4

Allelopathic effects of exotic invasive and native trees on co-existing undercanopy species. The soil as modulator

Qué pequeña eres brizna de hierba! Si, pero tengo toda la Tierra a mis pies.

Rabindranath Tagore

Este capítulo reproduce el texto del siguiente manuscrito:

This chapter reproduces the text of the following manuscript:

Medina-Villar S, Alonso A, Castro-Díez P, Pérez-Corona E. Allelopathic effects of exotic invasive and native trees on co-existing undercanopy species. The soil as modulator. (En revisión en la revista Plant and Soil)

Fotografía: Hojas senescentes de *Populus alba* en agua destilada durante un día. Tras retirar las hojas y filtrar obtuvimos el extracto acuoso cuyo potencial alelopático fue testado sobre la germinación de diversas especies de plantas en este estudio.
Por: Silvia Medina Villar

Photography: Senescent leaves from *Populus alba* in deionized water for one day. After taking out the leaves we obtained the aqueous extract whose allelopathic potential was tested over the germination of several plant species in this study.
By: Silvia Medina Villar

El dibujo de los encabezados está modificado de Cierjacks et al. (2013).

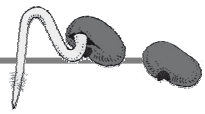
Picture of headers is a modification from Cierjacks et al. (2013)

Resumen

Efectos alelopáticos de los árboles exóticos invasores y nativos sobre las especies co-existentes del sotobosque. El suelo como modulador

Nuestro objetivo fue comparar los efectos alelopáticos de dos especies de árboles invasores (*Ailanthus altissima* (Mill.) Swngle y *Robinia pseudoacacia* L.) y dos nativos (*Fraxinus angustifolia* Vahl. y *Populus alba* L.) sobre varias especies de plantas del sotobosque, así como establecer la capacidad del suelo para modular dichos efectos alelopáticos. El efecto de los extractos acuosos de hojas senescentes de las especies arbóreas (denominadas especies donadoras), a concentraciones que imitan aquellas que podrían encontrarse en el campo, fue testado sobre: 1) el porcentaje de germinación (G) y el índice de velocidad de germinación (SGI) de las semillas de 13 especies del sotobosque (denominadas especies diana) usando papel de germinación (GP) como sustrato (Primer bioensayo) y 2) el G, el SGI y el crecimiento de radícula de 6 especies diana usando suelo de la orilla del río y GP como sustratos (Segundo bioensayo). Nuestros resultados mostraron que las especies exóticas no produjeron mayores efectos alelopáticos que las nativas. Encontramos efectos especie-específicos entre especies donadoras y especies diana, siendo *R. pseudoacacia* la especie más alelopática y *Trifolium repens* L. la especie diana más sensible en GP. El suelo redujo enormemente los efectos alelopáticos encontrados en GP. Los efectos especie-específicos pueden implicar una diferente composición de la comunidad de plantas bajo el dosel de las especies arbóreas estudiadas. Sin embargo, la extrapolación de los experimentos alelopáticos de laboratorio sobre GP a las condiciones naturales en el campo resulta complicada debido a la fuerte habilidad del suelo de la orilla del río para reducir los efectos alelopáticos.

Palabras clave: *Ailanthus altissima*; Fitotoxicidad; *Fraxinus angustifolia;* impactos; *Populus alba; Robinia pseudoacacia.*



Abstract

Allelopathic effects of exotic invasive and native trees on co-existing undercanopy species. The soil as modulator

We aimed to compare the allelopathic effects of exotic invasive tree species (*Ailanthus altissima* (Mill.) Swngle and *Robinia pseudoacacia* L.) with those of natives (*Fraxinus angustifolia* Vahl. and *Populus alba* L.) on the fitness of several undercanopy plant species and to assess the capacity of soil to modulate these allelopathic effects. Aqueous leaf litter extracts from the tree species (called as donor species) at concentrations mimicking those we can find in the field were tested: 1) on the percentage of seed germination (G) and on the speed germination index (SGI) of 13 undercanopy species (called as target species) using germination paper (GP) as substratum (First bioassay) and 2) on G, SGI and radicle growth of 6 target species using both river bank soil and GP as substratum (Second bioassay). Exotic species did not produce greater allelopathic effects than natives. We found species-specific effects between donor and target species being *R. pseudoacacia* the most allelopathic species and *Trifolium repens* the most sensitive target species in GP. Soil greatly reduced the allelopathic effects found in GP. The species-specific effects may imply different plant community composition under the canopy of each tree species due to allelopathy. However, the extrapolation of lab-experiments to natural field conditions is complicated due to the strong ability of the river bank soil to reduce the allelopathic effects.

Key words: *Ailanthus altissima*; *Fraxinus angustifolia;* impacts; Phytotoxicity; *Populus alba; Robinia pseudoacacia.*

Introduction

Allelopathy is a phenomenon by which certain chemical compounds of some plant species (called donor species) can reduce the fitness of other species (called target species) (Rice 1984). These chemical compounds, also referred as allelopathic or allelochemical compounds, can be released as root exudates, volatilized from leaf stomata, derived from litter decomposition or leached out from different dead or living plant organs during rainfall events (Rice 1984; Kobayashi 2004). The study of allelopathy as a competitive strategy in plant interactions has gained renewed interest in the context of exotic plant invasions, as it can explain the ability of some exotic invasive plant species (EIPS) to virtually form monocultures (Bais et al. 2003; Hierro and Callaway 2003). In fact, allelopathy has been considered as one of the mechanisms contributing to the invasion success of some EIPS, as stated in *The Novel Weapons Hypothesis* (NWH) (Callaway and Ridenour 2004), and also as a mechanism of impact (Skurski et al. 2014). According to NWH, some exotic plants successfully invade ecosystems and cause more negative effects in the invaded than in the native range because the species of the invaded range are not adapted to the novel chemical compounds (Callaway and Ridenour 2004; Callaway et al. 2008; Thorpe et al. 2009). In fact, chemical compounds found in EIPS were documented in less species than those found in exotic not-invasive plants (Cappuccino and Arnason 2006). Besides, allelopathic compounds from EIPS can indirectly affect the growth of native plant species by depressing the abundance of their mutualistic microbes, such as mycorrhyzal fungi (Callaway et al. 2008; Grove et al. 2012). This indirect effect may persist for years after the elimination of the EIPS (Grove et al. 2012).

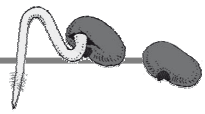
Several studies compared the allelopathic potential of EIPS in the native and invaded range (e.g. Thorpe et al. 2009), but comparisons between allelopathic potential of co-existing EIPS and native plant species are poorly explored. However, native plants can also interact among them by means of allelopathy similarly or even with more effectiveness than the EIPS with the

native species (Catalán et al. 2013; Del Fabbro and Prati 2015; Shannon-Firestone and Firestone 2015). Therefore, in order to relativize the allelopathic impacts of EIPS on native species it is relevant to study allelopathic effects produced by EIPS in comparison with those produced by native plant species over the same target species. On the one hand, to consider allelopathy as a mechanism of impact by EIPS, their allelopathic effects on target species of the invaded ecosystem should be greater than those produced by native species. On the other hand, if allelopathic effects produced by EIPS and native species were similar, allelopathy act as a general mechanism of dominance by both exotic and native plant species (Kim and Lee 2011; Meiners et al. 2012; Del Fabbro and Prati 2015).

Many scientists have been very critical and skeptic with allelopathy as a mechanism of plant-plant interactions because it is usually tested under laboratory unrealistic conditions (Inderjit and Weiner 2001; Meiners et al. 2012). For instance, many studies assessed allelopathic potential in laboratory bioassays using high concentrations of plant extracts, filter paper as germination substratum (e.g. Pisula and Meiners 2010; Catalán et al. 2013) and model or assays plant species (i.e. phytometers) as target species, which were selected by their rapid germination and high sensitivity to a wide range of allelopathic compounds (e.g. De Feo et al. 2003; Kaur et al 2009; Pisula and Meiners 2010). Therefore, to assess the ecological relevance of allelopathy, the experimental conditions should mimic field conditions and use a wide range of target species co-existing in the recipient ecosystem with the invaders (Hierro and Callaway 2003; Inderjit and Nilsen 2003). In addition, the allelopathic effects in the field can be greatly affected by the soil characteristics because of the multiple interactions between allelochemicals and soil components (Inderjit and Weiner 2001; Kobayashi 2004; Kaur et al. 2009). For instance, the microbiological activity of soils can modify the chemical structure and/or activity of the allelopathic compounds, reducing or enhancing their effects on target species (Kobayashi 2004; Inderjit and Van der Putten 2010; Cipollini et al.

2012). Soil nutrients may also mask the allelopathic effects on plant growth (Blum et al. 1993) and soil organic matter (SOM) usually absorbs phenolic acids reducing their activity (Pollock et al. 2009). Finally, soil texture acts as a relevant factor modulating the effects of allelopathic compounds, which were usually greater in sandy soils (Oleszek and Jurzysta 1987; El-Darier et al 2014) likely due to their poor capability to absorb chemical compounds.

Ailanthus altissima (Mill.) Swingle (Simaroubaceae) and *Robinia pseudoacacia* L. (Fabaceae) are two aggressive EIPS distributed worldwide in temperate zones (Kowarik and Säumel 2007; Cierjacks et al. 2013) being both considered within the 100 worst invasive species in Europe and within the 20 most harmful EI species in Spain (GEIB 2006; DAISIE 2009). They usually invade road and crop borders, as well as riparian areas where they co-exist with native trees, such as *Populus alba* L. (Salicaceae) and *Fraxinus angustifolia* Vahl. (Oleaceae) in Spain (Sanz-Elorza et al. 2004, Castro-Díez et al. 2014d). These EIPS may alter leaf litter decomposition both in soil and water, as well as the structure of decomposer communities (Alonso et al. 2010; Castro-Díez et al. 2012; Gutiérrez-López et al. 2014; Medina-Villar et al. 2015b). Both *A. altissima* and *R. pseudoacacia* have chemical compounds in their tissues with recognized allelopathic effect on some target species (Heisey 1990; Heisey and Heisey 2003; De Feo et al. 2003; Nasir et al. 2005; Catalán et al. 2013). Besides, allelopathy has been suggested as a mechanism explaining the success of *A. altissima* (Lawrence et al. 1991; Heisey and Heisey 2003; Gómez-Aparicio and Canham 2008a) and *R. pseudoacacia* (Nasir et al. 2005). However, the allelopathic effects of both species, as compared to those of co-existing native trees have not been explored under realistic conditions. Thus, our study aims to evaluate the importance of allelopathy as a mechanism of plant impact by comparing the effects of leaf litter extracts of exotic invasive (*A. altissima* and *R. pseudoacacia*) and native (*P. alba* and *R. pseudoacacia*) tree species on a wide range of undercanopy target species co-ocurring with them. We monitored different stages of plant growth, mimicked realistic concentrations of leaf extracts and used natural soil as



substratum. More specifically, we addressed the following questions: 1) Do leaf litter extracts of native and EIPS cause allelopatic effects on several undercanopy species at environmental realistic leaf extract concentrations? 2) Have the EI trees higher allelopathic potential than co-existing native trees? 3) What target species and what fitness indicators are the most sensitive to allelopathy of the studied species? 4) How does the soil modify the allelopatic effects of donor species? Based on the NWH (Callaway and Ridenour 2004) we expected greater allelopathic effects produced by the EIPS than those produced by natives, because target species are not adapted to the allelochemicals from the EIPS. We also expected soil to mitigate the allelopathic effects due to its capacity to degrade or to absorb the allelopathic compounds.

Materials and Methods

Species selection

The donor species were two EIPS (*A. altissima* and *R. pseudoacacia*) and two native species (*F. angustifolia* and *P. alba*) co-occurring in riparian areas of the Henares River (Central Spain) (Castro-Díez et al. 2014d). The target species were 13 native plants also co-occurring with donor plants in riparian areas of Henares River (Martínez 2000) (Table 1). Two target species were two of the donor trees, *R. pseudoacacia* and *P. alba*, which allowed testing for auto-allelopathy, and the remaining 11 target species were herbs. Target species were chosen to fulfill a high variety of growth forms (phanerophytes, hemicryptophytes and therophytes) and life strategies (annual, biennial and perennial) (Table 1). In addition, target species had high and fast rates of germination, which make them suitable to perform germination bioassays. Other potential target species appearing in floodplains of the Henares River were discarded because of their low germination success (unpublished results). Seeds from herbaceous species were commercially obtained from *Semillas Silvestres* S.L. Seeds from *R. pseudoacacia* and *P. alba* were collected in the field from at least 5 trees in a riparian area near Alcalá de Henares, Madrid, Spain (3

° 20´ W, 40 ° 29´ N). Seeds from *R. pseudoacacia* were collected on March 2012 and conserved in plastic bags in the fridge at 4º C before use. *P. alba* seeds were collected twice, on the month of May 2013 and 2015, and immediately used before one week, which is the approximate time frame for which these seeds remain viable (Prada and Arizpe 2008).

Leaf material

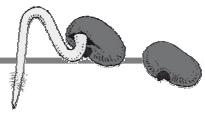
We used leaves to prepare aqueous leaf litter extracts (called "leaf extracts" through the whole manuscript) since leaves represent the greatest fraction of the litter fallen in the soil in the studied riparian forests (Medina-Villar et al. 2015a). In autumn 2011, senescent leaves from donor species were collected from at least 5 trees per species in riparian areas near to Alcalá de Henares and Fresno del Torote (Madrid, Spain). To homogenize the material for the extracts we used leaves of *P. alba* and leaflets of the other donor species, which have compound leaves. However, we will refer to all of them as "leaves" throughout the whole manuscript.

Table 1. Family, life form, life span and group by stem tissue of the target species present in floodplains of the Henares River (Martínez 2000) used in the bioassays of this study.

Target species	Family	Life form	Life span	Group by stem tissue (Herbs/Woody)	Bioassays
Agrostis stolonifera L.	Poaceae	Hemicryptophytes	Perennials	Herbs	1
Bromus hordeaceus L	Poaceae	Therophytes	Annual or biennial	Herbs	1 and 2
Dactylis glomerata L.	Poaceae	Hemicryptophytes	Perennials	Herbs	1
Lolium rigidum L.	Poaceae	Therophytes	Annual	Herbs	1
Medicago sativa L.	Fabaceae	Hemicryptophytes	Perennials	Herbs	1 and 2
Trifolium repens L.	Fabaceae	Hemicryptophytes	Perennials	Herbs	1 and 2
Plantago lanceolata L.	Plantaginaceae	Hemicryptophytes	Perennials	Herbs	1 and 2
Urtica dioica L.	Urticaceae	Hemicryptophytes	Perennials	Herbs	1 and 2
Echium vulgare L.	Boraginaceae	Hemicryptophytes	Biennial	Herbs	1
Silybum marianum (L.) Gaertn	Asteraceae	Hemicryptophytes	Annual or biennial	Herbs	1
Chenopodium album L.	Amaranthaceae	Therophytes	Annual	Herbs	1
Populus alba L.	Salicaceae	Phanaerophytes	Perennials	Woody	1 and 2
Robinia pseudoacacia L.	Fabaceae	Phanaerophytes	Perennials	Woody	1

Preparation of leaf extracts

Two grams of air-dried leaves of each donor species were mixed up with 100 mL of deionized water to reach the leaf extract concentration of 20 g L^{-1}. The mixture was soaked in an orbital shaker (J.P. Selecta 3000974) at 90 rpm for 24 hours at room temperature. The solution was then filtered with a polyethersulfone membrane of 0.22 µm (Filtros ANOIA S.A. Barcelona, Spain) to eliminate organic matter, mineral particles, fungi spores, and most of the bacteria (Wang et al. 2007). For each donor species, the leaf extract of 20 g L^{-1} was diluted with deionized water to obtain decreasing concentrations (i.e. proportions of leaf litter to water) of 8.5 g L^{-1}, 2.5 g L^{-1} and 1 g L^{-1}. These concentrations aim to mimic those at which seeds are exposed in the field. They were selected within a range of likely concentrations, which could be reached in the field, and were assessed by two approximations: 1) dividing the total amount of leaf litter fallen each month (g m^{-2} $month^{-1}$) from June 2011 to December 2012 in several populations of the middle Henares River (data from Medina-Villar et al. 2015a), by the total precipitation of the same months (L m^{-2} $month^{-1}$), and 2) simulating a scenario in which the leaf litter accumulated each month (g m^{-2} $month^{-1}$) in the mentioned populations from June 2011 to December 2012 (Medina-Villar et al. 2015a) receives a unique and likely event of rainfall of 5 L m^{-2} day^{-1} in a single day, according to the register of diary precipitation of 2011-2012 in the rainfall stations of Guadalajara and Jadraque (http://sig.marm.es/saih/; Medina-Villar et al. 2015a). Following these approximations, seeds in soils under the canopy of all donor species could receive the leaf extracts concentrations of 1 g L^{-1}, 2.5 g L^{-1}, 8.5 g L^{-1} while the concentration of 20 g L^{-1} could be reached only under the canopy of *A. altissima* and *P. alba* (Fig. 1). However, considering precipitation events lower than 5 L m^{-2} day^{-1} or leaf litter accumulation during more than one month, the concentration of 20 g L^{-1} might be reached under the canopy of all donor species and significantly surpassed under *A. altissima* and *P. alba.* Although it is difficult to know the real concentrations of leaf litter lixiviates in the field (Inderjit and



Nilsen 2003), our calculations showed that the leaf litter/water proportion selected to obtain leaf extracts are in the range of what can be found in the field. Osmotic effects may affect seed germination and radicle growth of the target species, leading to overestimation of allelopathic effects (Wardle et al. 1992). To account for these potential osmotic effects of the extracts on the target variables, pH and conductivity of each leaf extract were measured with a pH-meter (Crison micropH 2001) and a conductivity-meter (Crison CM 35+), respectively (Online resource 1). The range of electrical conductivities of our extracts did not produced osmotic effects in a similar previous study (Escudero et al. 2000). The leaf extracts were conserved at 4 ^{o}C before use (not more than one week).

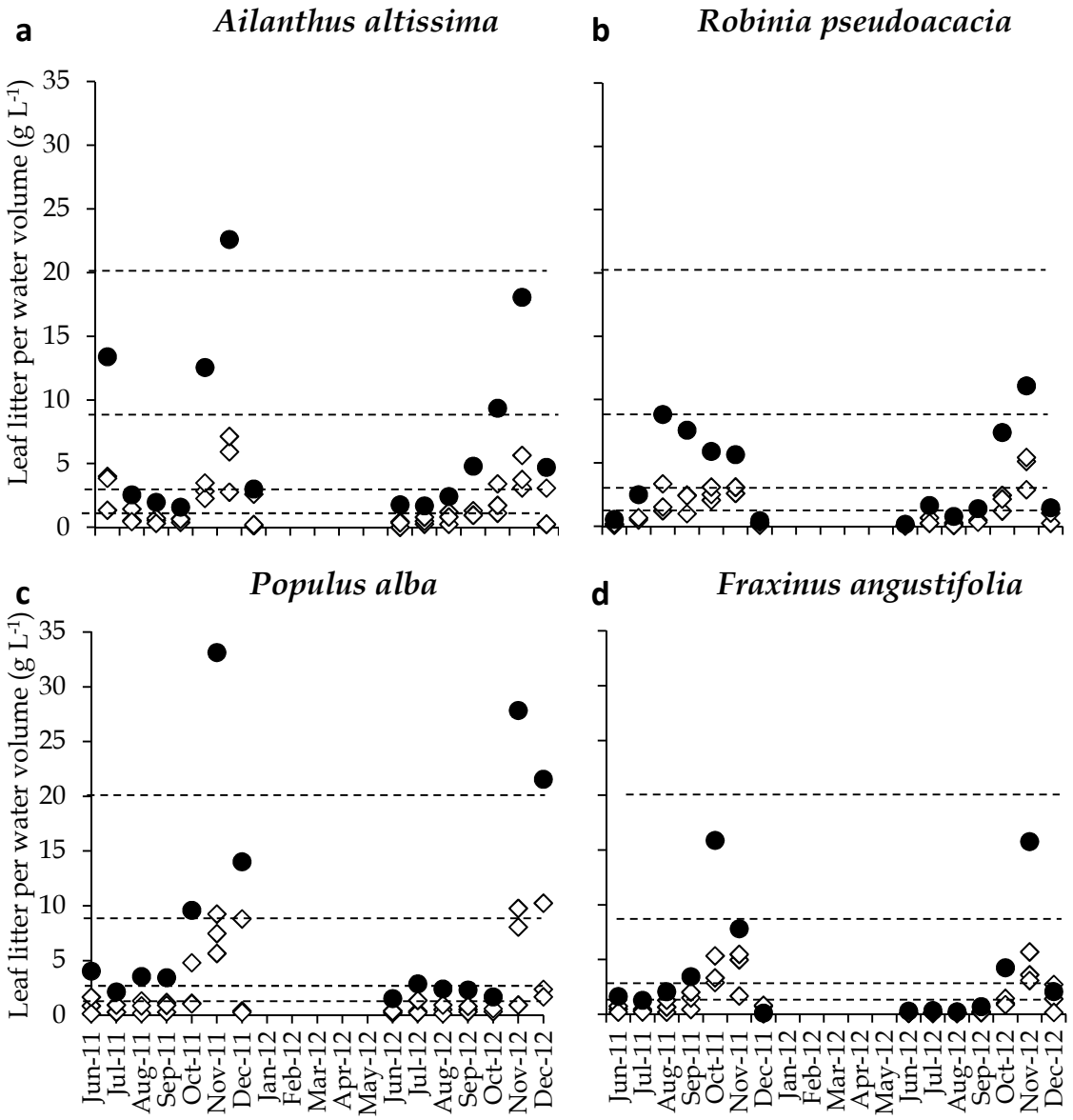
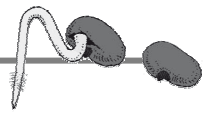


Figure 1. Total amount of leaf litter collected in three 300 m²-plots (using 18 litter traps per plot) dominated by the tree species *Ailanthus altissima* (**a**), *Robinia pseudoacacia* (**b**), *Populus alba* (**c**) and *Fraxinus angustifolia* (**d**) (data from Medina-Villar et al. 2015a) per total water volume fallen as rainfall each month from Jun 2011 to 2012 (g L^{-1}) (Data from rainfall stations of Guadalajara and Jadraque; http://sig.marm.es/saih/). White diamonds indicate for each month the average of these proportions (n = 3 plots) while the black circles indicate the average of total leaf litter (n = 3 plots) per water fallen in a single event of 5 L m^{-2}. Dashed lines indicate the proportions of leaf litter per water volume (g L^{-1}) used to obtain leaf litter extracts in the bioassays.



Bioassays

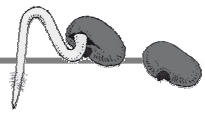
We performed two bioassays. The first one was performed between May 2013 and August 2014 and aimed to know the potential allelopathic effects of extracts with high-concentration (20 g L^{-1}) of the four donor species on the 13 target species and to rank donor species by their allelopathy and target species by their sensitivity. The aim of the second bioassay was two assess the role of soil as allelopathic modulator as well as the sensitivity of different fitness indicators of the target species to leaf extracts at decreasing concentrations. The second bioassay was performed between May 2014 and December 2015.

In the first bioassay, we tested the potential allelopathic effects of the 20 g L^{-1} leaf extracts of each donor species on the germination success (maximum percentage of germinated seeds) and on germination speed of the 13 target species (Table 1). Seeds of the target species were disinfected by submerging them in successive washes of bleach (50 %) and ethanol (96º) during 1 minute and finally rinsed with deionized water. Four ml of the 20 g L^{-1} extract from each donor species were applied to three-replicated 10 cm-diameter petri dishes containing germination paper (GP) as substratum (Filtros ANOIA S.A, Barcelona, Spain) and 20 seeds of one of the 13 target species. To make controls we added four ml of deionized water instead of leaf extracts. This gave a total of 15 petri dishes ((3 replicates x 4 donor species) plus 3 controls) per target species (Total: 195 petri dishes). Petri dishes were placed in a germination chamber at 24 ºC with a light/dark period of 12h/12h. We counted the number of seeds germinated each day until stabilization (no seed germination in 3 days) and calculated the percentage of seed germination (G). We considered that a seed was germinated when the radicle was roughly 1 mm. Seeds germinated each day were removed from petri dishes. The speed germination index (SGI) was calculated following Wardle et al. (1991):

$$SGI = \left[N1 + \frac{N2}{2} + \frac{N3}{3} + \cdots + \frac{Nn}{n}\right] x\ 100$$

being, N_1, N_2, N_3, N_n, the proportion of seeds germinated in days 1, 2, 3, n, from the beginning of the bioassay. This index varies from 0 (if any seed germinate at the end of the bioassay) to 100 (if all seeds germinate the first day).

In the second bioassay we selected the six target species which were affected by at least one leaf extract in the first bioassay ($P < 0.05$ and exceptionally $P < 0.07$) and belonging to different families (Table 1). Different concentrations of the leaf extracts (20 g L^{-1}, 8.5 g L^{-1}, 2.5 g L^{-1} and 1 g L^{-1}) were tested using G, SGI and radicle growth as indicators of fitness. We used GP and soil as substratums. The soil was collected on June 2013 and November 2014 from a native forest area dominated by the native tree *P. alba* in Guadalajara, Central Spain (40° 43′ N, 3° 9′ W). Soil was homogenized, dried at room temperature during one week, sieved (2 mm mesh) and preserved before use (16 months maximum) in a cardboard box at 4 ºC. The soil amount used in each petri dish was 15g. The soil was Calcic Cambisol + Calcic regosol + Chromic luvisol with a fine texture, moderately well drainage, 19 % sand, 32 % silt, 49 % clay (Monturiol and Alcalá del Olmo 1990; FAO 2012). In a previous study we found that the soil has 0.22 % of total N, 0.04 % of total P, 4.95 % organic matter and pH of 7.9 (Medina-Villar et al. 2015a). The procedure and experimental conditions to assess allelopathic effects on G and SGI were similar to those we explained above for the first bioassay. To evaluate the allelopathic effects of the leaf extracts on G and SGI of each target species, a total of 170 petri dishes (5 replicates x 4 donor species x 4 extract concentrations x 2 substratums) plus the control plates with deionized water, 5 in soil substratum and 5 in GP) were used (Total: 1020 petri dishes). Twenty disinfected seeds per target species and petri dish were used. To measure the radicle growth a pool of disinfected seeds was placed in plates with GP, and then introduced in the germination chamber (24 ºC, 12h/12h light/dark). We selected 20 recently-germinated seeds (i.e.



those with similar radicle length, less than 2 mm, or 1mm if the species showed slow radicle growth) of each target species and they were placed on each of the 102 petri dishes ((3 replicates x 4 donor species x 4 extract concentrations x 2 substratums) plus controls with deionized water, 3 in soil substratum and 3 in GP) (Total: 612 petri dishes). Petri dishes were reintroduced in the germination chamber and after 24h, seeds and seedlings were scanned (Aficio MP C4501 RICOH). Radicle length was measured from digital images (.GIF) using the segmented line tool in the software imageJ (Rasband 1997-2015; http://imagej.nih.gov/ij/).

Statistical analyses

We standardized the values of fitness indicator variables (G, SGI and radicle length) for each target species as:

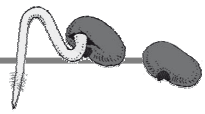
$$\frac{(C - x)}{C}$$

where x is each value of G, SGI and radicle length and C is the mean value of controls. Standardized variables were used to measure the magnitude of the effects produced by the treatments with respect to the controls. Greater values of standardized variables indicate greater effectiveness of the leaf extracts to reduce the fitness indicator regarding controls (i.e. as the case may be greater sensitiveness of target species to leaf extracts).

For the first bioassay, one way ANOVA followed by Dunnett test was performed to assess differences in the G and SGI of each target species between controls and the leaf extracts from different donor species at 20 g L^{-1}. The number of significant effects on G and SGI (ANOVA, Dunnett test, $P < 0.05$) were counted to order the target and donor species by the frequency of effects received and produced, respectively. Differences in standardized G and SGI among target species and among donor species were assessed by two-way ANOVA (target species x donor species) followed by Tukey-HSD test. This allowed us to statistically sort both target species by their sensitivity to leaf

extracts and donor species by their effectiveness to reduce G or SGI of the target species throw leaf extracts.

For each of the six target species selected in the first bioassay, a three-way ANOVA followed by Tukey-HSD test was performed to assess the effects of germination substratum (soil or GP), donor species and the concentration of the leaf extracts on G, SGI and radicle length. Controls were considered as a level of extract concentration (i.e. 0 g L^{-1}). We performed a contingency analysis (Fisher´s exact test, Quinn and Keough 2002) to identify if significant effects produced by leaf extracts from EIPS on G, SGI and radicle length of the target species were more frequent than those produced by leaf extracts from native species. For this purpose we counted the number of significant effects (ANOVA, Tukey HSD, $P < 0.05$) produced by leaf extracts of EIPS and native species in the bioassay 2, considering only significant differences between leaf extracts and control. To assess differences in standardized G, SGI and radicle length both among target species and donor species in each substratum, a four-way ANOVA (target species x substratum x donor species x concentration) followed by Tukey-HSD test was performed. R software 3.1.2 (R Core Team, 2014) was used for three and four-way ANOVA and JMP, Version 7 (SAS Institute Inc., Cary, NC, 1989-2007) was used for the rest of analyses.



Results

First bioassay

Nine out of the 13 target tested species responded to any of the leaf extracts in GP substratum (Fig. 2, $P < 0.05$). Leaf extracts of all donor species reduced G of *P. lanceolata* and the SGI of *A. stolonifera*, *B. hordeaceus*, *P. alba, P. lanceolata* and *T. repens* (Fig. 2, Dunnett test, $P < 0.05$). However, other target species were sensitive to only part of the donor. For instance, only *P. alba* and *R. pseudoacacia* leaf extracts reduced G of *T. repens* (Fig. 2a, Dunnett test, $P < 0.05$); *F. angustifolia* was the only species whose leaf extracts reduced the SGI of *U. dioica* and *C. album* (Fig. 2b, Dunnett test, $P < 0.05$); and the *A. altissima* leaf extract was the only that effectively reduced SGI of *L. rigidum* (Fig. 2b, Dunnett test, $P < 0.05$). Lastly, only leaf extracts of *P. alba* reduced the SGI of *M. sativa,* although differences were marginally significant (Fig. 2b, Dunnett test, $P = 0.070$). SGI was more affected by leaf extracts than G, since the SGI of nine target species responded to leaf extracts while only in two target species G responded to leaf extracts (Fig. 2, Dunnett test, $P < 0.05$).

Leaf extracts from EIPS did not produce more significant effects than those from native species (14 vs. 16) (Fig. 2, Dunnett test, $P < 0.05$). In addition, according to the magnitude of the effects (as showed by the standardized G and SGI), EIPS were not more effective against target species than natives, being only the *P. alba* more effective than *A. altissima* reducing the SGI of the target species (Tukey HSD test, $P < 0.05$) (Data not shown). We ordered the target species by the number of significant effects that G and SGI showed after the exposure to leaf extracts (Fig. 2, Dunnett test, $P < 0.05$) as: *P. lanceolata* (8 effects) > *T. repens* (6 effects) > *B. hordeaceus* = *P. alba* = *A. stolonifera* (4 effects) > *M. sativa* = *U. dioica* = *L. rigidum* = *C. album* (1 effect). Besides, according to the magnitude of the effects on G and SGI, we found that the most sensitive species were *T. repens* and *A. stolonifera* (Fig. 3, Tukey HSD test, $P < 0.05$).

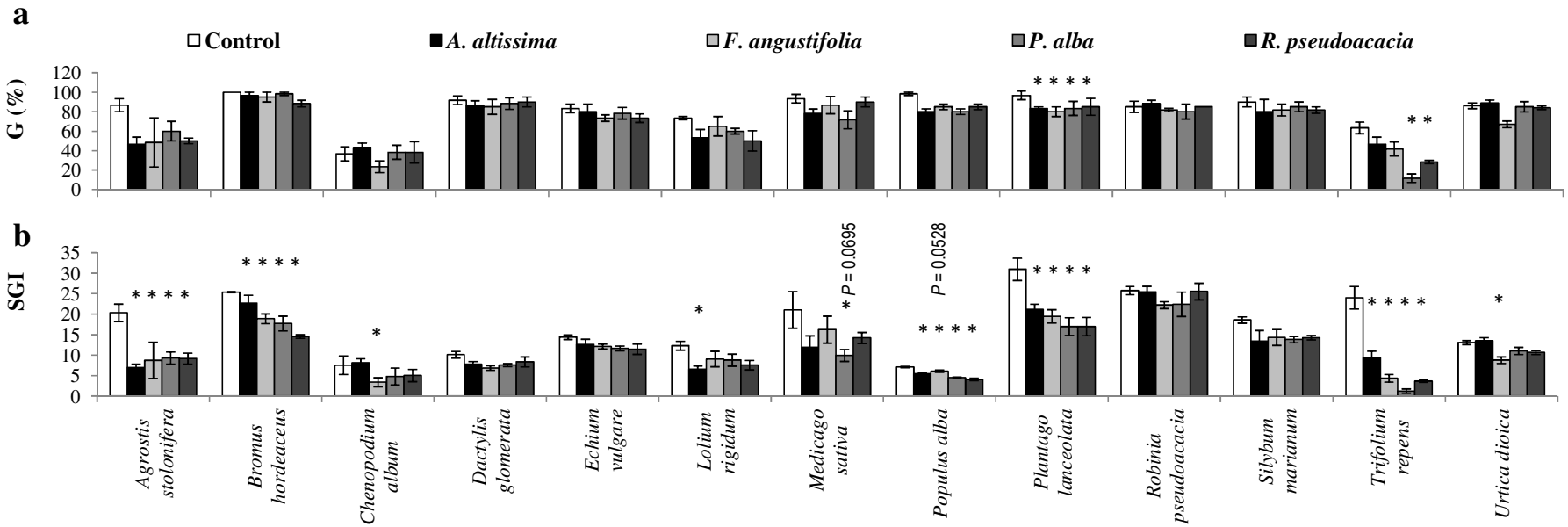


Figure 2. First bioassay: Percentage of seed germination (G) (Mean ± SE, n = 3) (a) and speed germination index (SGI) (Mean ± SE, n = 3) (b) of seeds from target species submitted to deionized water (Control) and aqueous leaf litter extracts from donor species (*Ailanthus altissima*, *Fraxinus angustifolia*, *Populus alba* and *Robinia pseudoacacia*) at the concentration of 20 g L^{-1} using germination paper as substratum. Asterisks mean significant differences between the control and each donor species (Dunnett test, $P < 0.05$). When differences are marginally significant (P 0.05-0.07) the P value is showed.

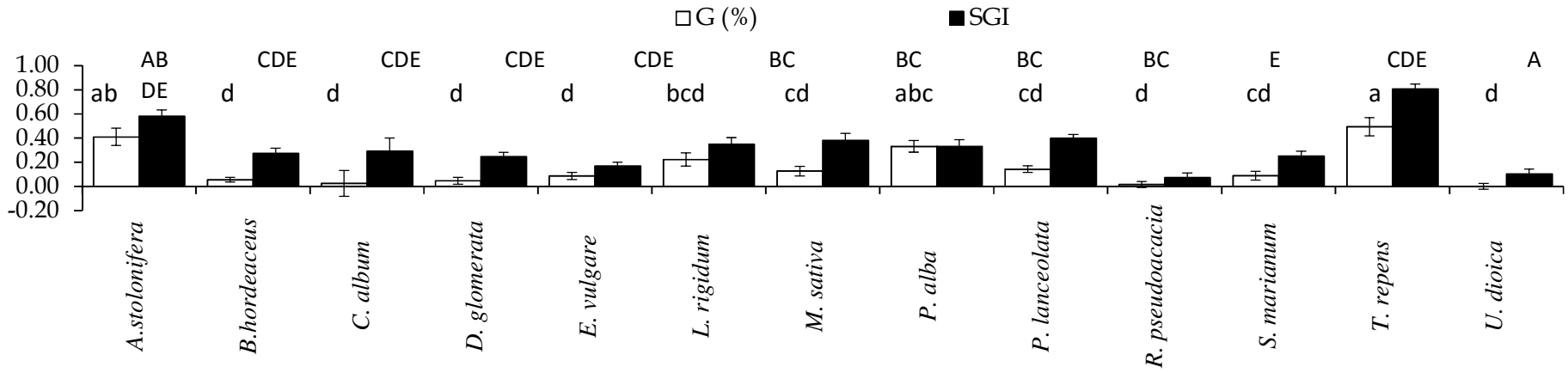


Figure 3. First bioassay: Mean (±SE, n = 4) of the standardized percentage of seed germination (G (%)) (white bars) and speed germination index (SGI) (black bars) of the target species (*Agrostis stolonifera*, *Bromus hordeaceus*, *Chenopodium album*, *Dactylis glomerata*, *Echium vulgare*, *Lolium rigidum*, *Medicago sativa*, *Populus alba*, *Plantago lanceolata*, *Robinia pseudoacacia*, *Silybum marianum*, *Trifolium repens*, *Urtica dioica*) in presence of the leaf litter extracts from the different donor species (*Ailanthus altissima*, *Fraxinus angustifolia*, *Populus alba* and *Robinia pseudoacacia*) at the concentration of 20 g L^{-1} using germination paper as substrate. Different letters mean significant differences of G (%) (lower case letters) and SGI (uppercase letters) among target species (Tukey HSD, $P < 0.05$).

Second bioassay

Among the species significantly affected by the leaf extracts in first bioassay, we selected six species belonging to different families: Fabaceae (*T. repens* and *M. sativa*), Poaceae (*B. hordeaceus*), Plantaginaceae (*P. lanceolata*), Urticaceae (*U. dioica*) and Salicaceae (*P. alba*) to perform the second bioassay (Table 1). Substratum, donor species and the concentration of leaf extracts affected G, SGI and radicle length of the target species (Online resource 2, three-way ANOVA, $P < 0.05$). Several interactions among these factors were also significant (Online resource 2; three-way ANOVA, $P < 0.05$). Over GP substratum, only one target species (*T. repens*) reduced its germination in presence of at least one concentration of all donor species leaf extracts (Fig. 4, Tukey HSD, $P < 0.05$). In addition, some species-specific effects between donor and target species were found in SGI and radicle length (Figs. 5 and 6). For instance, SGI of *P. lanceolata* was reduced by *R. pseudoacacia* and *P. alba* but not by the other donor species (Fig. 5, Tukey HSD, $P < 0.05$) and the radicle growth of *P. alba* and *P. lanceolata* was reduced by leaf extracts of all donor species except by *F. angustifolia* (Fig. 6, Tukey HSD, $P < 0.05$). Radicle length was the most sensitive indicator since it was negatively affected in all target species by leaf extracts from at least one of the donor species (Fig. 6, Tukey HSD, $P < 0.05$,). We found many effects of leaf extracts either on G, SGI or radicle length of all donor species (with respect to controls) when GP was used as substratum. By contrast, when soil was used as substratum, only the radicle length of *U. dioica* was clearly reduced by all of the donor species leaf extracts (Online resource 2, Figs. 4, 5 and 6).

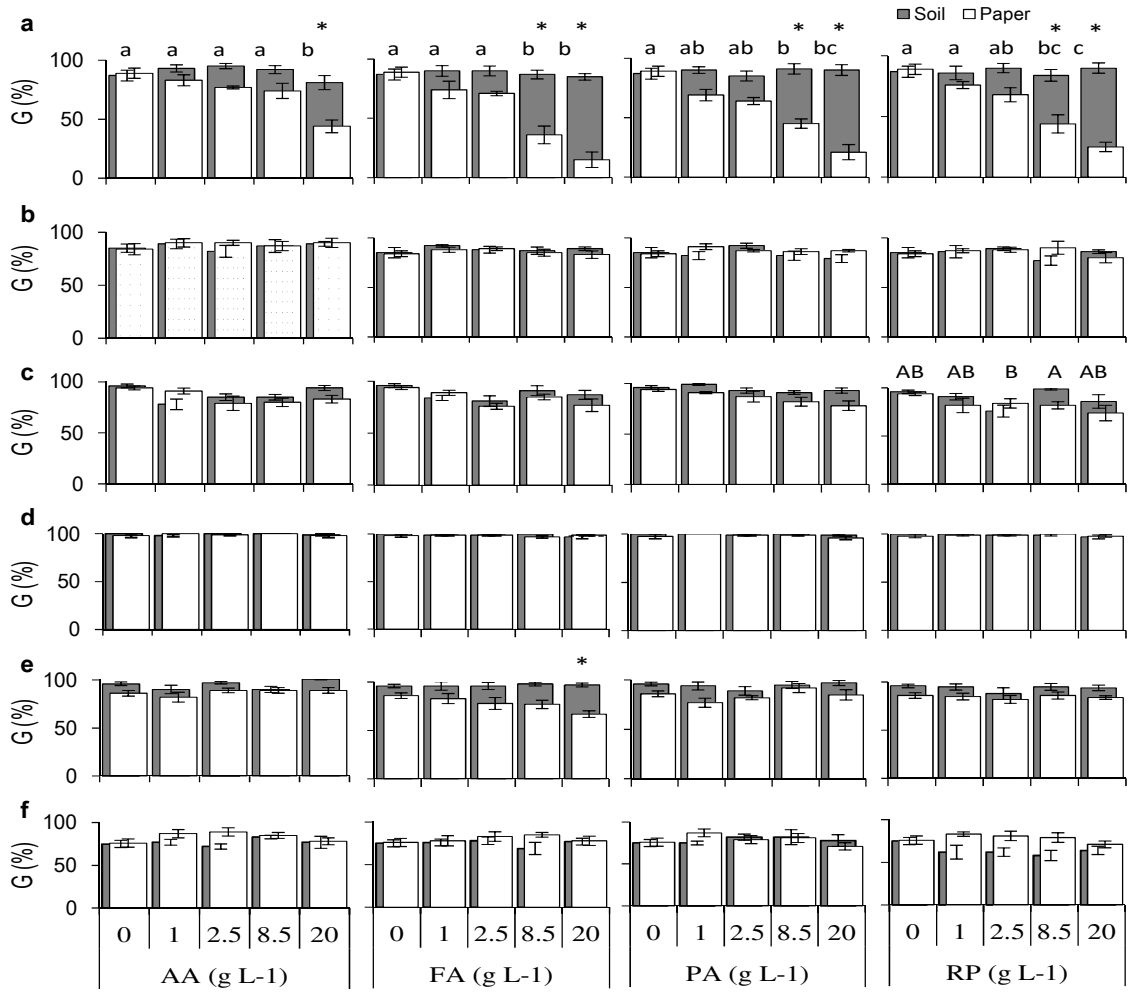


Figure 4. Second bioassay: Mean (± SE, n = 5) percentage of seed germination (G) of *Trifolium repens* (a), *Medicago sativa* (b), *Plantago lanceolata* (c), *Bromus hordeaceus* (d), *Urtica dioica* (e) and *Populus alba* (f) submitted to different concentrations (0, 1, 2.5, 8.5 and 20 g L^{-1}) of aqueous leaf extracts from *Ailanthus altissima* (AA), *Fraxinus angustifolia* (FA), *Populus alba* (PA) and *Robinia pseudoacacia* (RP). Grey and white bars diferentiate the substratum used: paper (white bars) or natural soil (grey bars, behind white ones). Dashed lines indicate the mean of grey bars when they are masked by the front white bars. Different letters mean significant differences in G among concentrations of each leaf extracts in paper substratum (lowercase letters) or soil substratum (uppercase letters) (ANOVA, Tukey HSD, $P < 0.05$). For each concentration of each leaf extract, asterisks indicate significant differences in G between substratums (paper and soil) (ANOVA, Tukey HSD, $P < 0.05$).

Similarly to the first bioassay we did not find greater number of negative effects of leaf extracts from EIPS as a group compared with those of natives (one tail Fisher´s exact test, $P = 0.10$), although leaf extracts from EIPS tended to produce more negative effects (Figs. 4, 5 and 6, Tukey HSD, $P < 0.05$) than those from natives (53 vs. 42 effects, considering those on G, SGI and radicle length, all extract concentrations an both substratums). Thus, the ranking of the donor

species by their number of significant effects (Tukey HSD, $P < 0.05$) reducing G, SGI and radicle length of the target species was: *R. pseudoacacia* (29 effects) > *P. alba* (25 effects) > *A. altissima* (24 effects) > *F. angustifolia* (17 effects) (Figs. 4, 5 and 6).

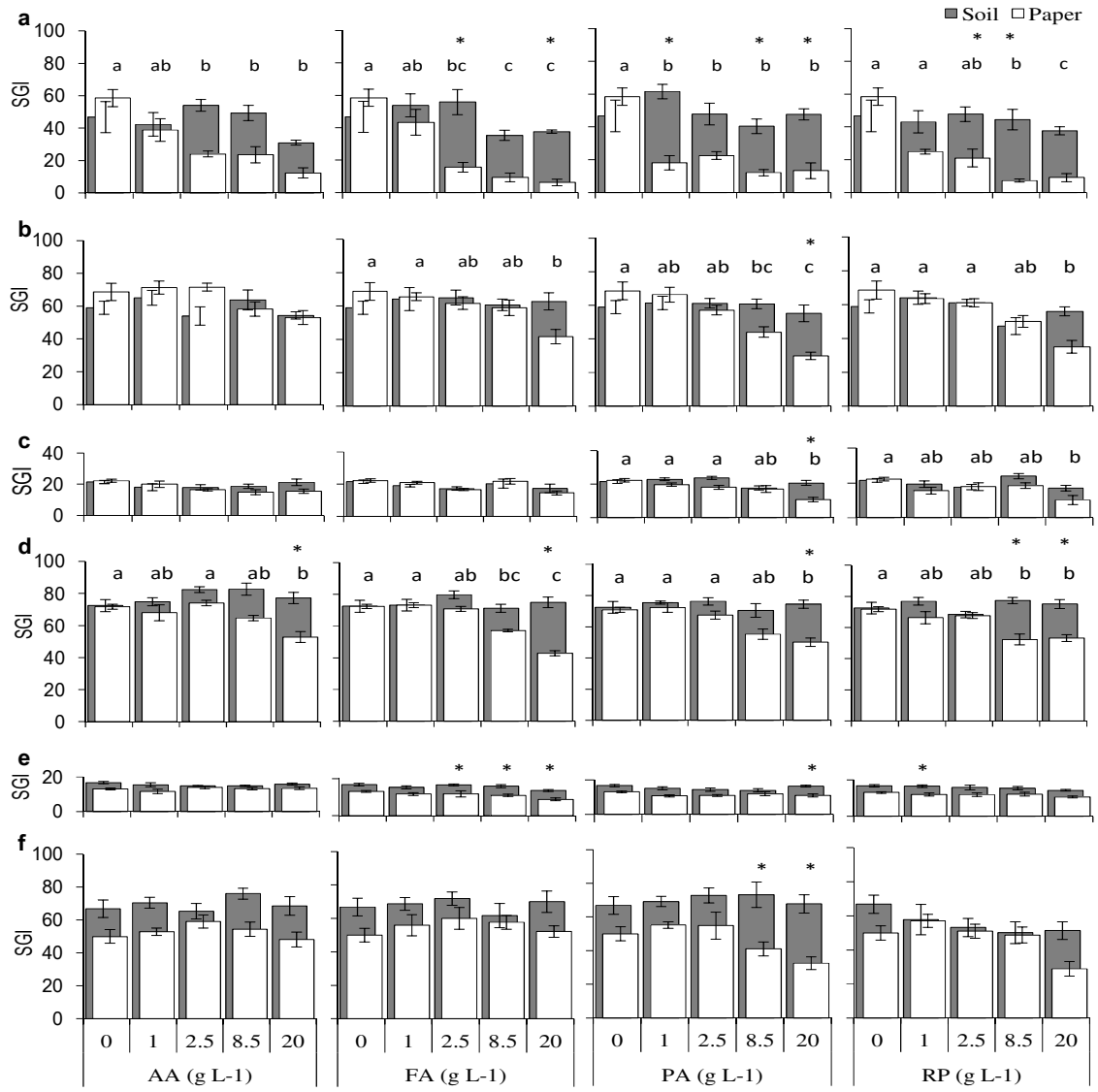


Figure 5. Second bioassay: Mean (± SE, n = 5) speed germination index (SGI) of *Trifolium repens* (a), *Medicago sativa* (b), *Plantago lanceolata* (c), *Bromus hordeaceus* (d), *Urtica dioica* (e) and *Populus alba* (f) submitted to different concentrations (0, 1, 2.5, 8.5 and 20 g L^{-1}) of aqueous leaf extracts from *Ailanthus altissima* (AA), *Fraxinus angustifolia* (FA), *Populus alba* (PA) and *Robinia pseudoacacia* (RP). Grey and white bars diferentiate the substratum used: paper (white bars) or natural soil (grey bars). Different lowercase letters mean significant differences in SGI among concentrations of each leaf extracts in paper substratum (ANOVA, Tukey HSD, $P < 0.05$). No differences were found in soil substratum. For each concentration of each leaf extract, asterisks indicate significant differences in SGI between substratums (paper and soil) (ANOVA, Tukey HSD, $P < 0.05$).

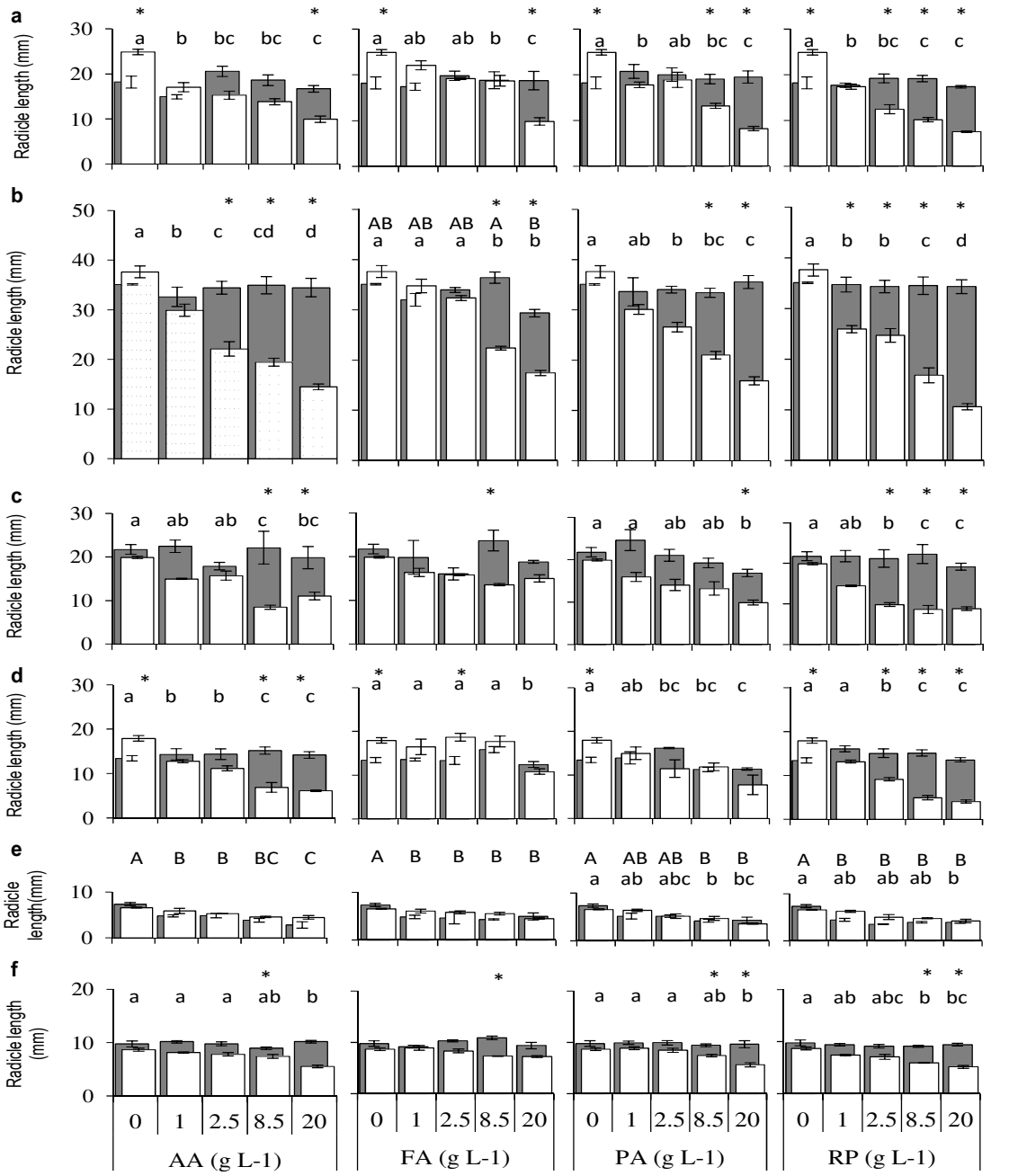


Figure 6. Second bioassay: Mean (± SE, n = 5) radicle length of the species *Trifolium repens* (a), *Medicago sativa* (b), *Plantago lanceolata* (c), *Bromus hordeaceus* (d), *Urtica dioica* (e) and *Populus alba* (g) in presence of different concentrations (0, 1, 2.5, 8.5 and 20 g L^{-1}) of aqueous leaf extracts from *Ailanthus altissima* (AA), *Fraxinus angustifolia* (FA), *Populus alba* (PA) and *Robinia pseudoacacia* (RP). Grey and white bars diferentiate the substratum used: paper (white bars) or natural soil (grey bars). Different letters mean significant differences in radicle length among concentrations of each leaf extracts in paper substratum (lowercase letters) or soil substratum (uppercase letters) (ANOVA, Tukey HSD, $P < 0.05$). For each concentration of each leaf extract, asterisks indicate significant differences in radicle length between substratums (paper and soil) (ANOVA, Tukey HSD, $P < 0.05$).

Regarding the magnitude of the effects produced by the donor species leaf extracts, *R. pseudoacacia* was the most effective reducing the radicle growth of the target species over GP substratum and the G and SGI of target species over soil substratum (Fig. 7 a and b, Tukey HSD, $P < 0.05$). Both *P. alba* and *R. pseudoacacia* reduced the SGI of the target species in paper substratum more effectively than the other donor species (Fig. 7 b, Tukey HSD, $P < 0.05$).

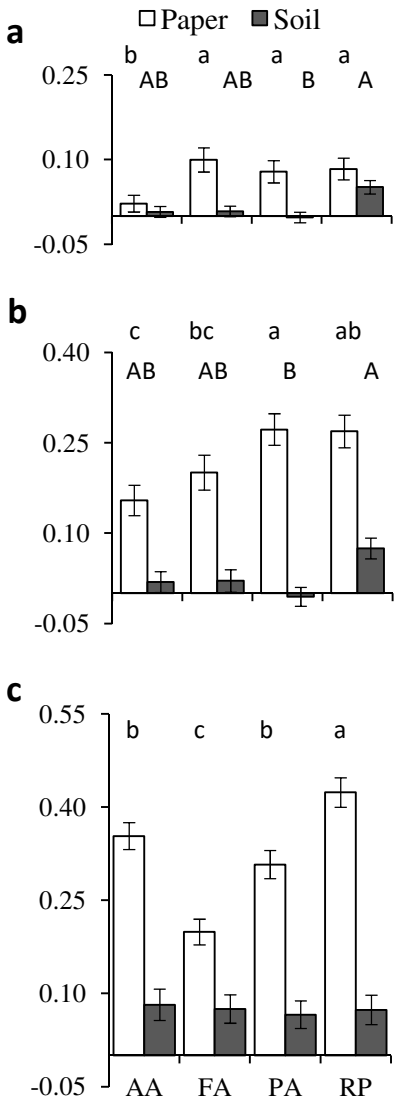


Figure 7. Second bioassay: Mean (±SE, n = 24) standardized differences between mean controls and the fitness indicators: percentage of seed germination (a), speed germination index (b) and radicle length (c) across all the target species submitted to leaf extracts of the donor species: *Ailanthus altissima* (AA), *Fraxinus angustifolia* (FA), *Populus alba* (PA) and *Robinia pseudoacacia* (RP) in paper (white bars) and soil (gray bars) substratum. Different letters mean significant differences among donor species in paper (lowercase letters) and soil (uppercase letters) as substratum (Tukey HSD, $P < 0.05$).

Finally, on paper substratum, the least effective leaf extracts were *A. altissima* reducing G and SGI of the target species and *F. angustifolia* reducing the radicle growth of the target species (Fig. 7, Tukey HSD, $P < 0.05$). The ranking of the target species according to the times that their G, SGI and radicle growth were significantly reduced (Tukey HSD, $P < 0.05$) by leaf extracts (regarding controls) was: *T. repens* (32 effects) > *M. sativa* = *B. hordeaceus* = *U. dioica* (17 effects) > *P. lanceolata* (8 effects) > *P. alba* (4 effects). In addition, according to the magnitude of the effects, *T. repens* was the most sensitive species in GP substratum and *U. dioica* the most sensitive in soil substratum (Fig. 8, Tukey HSD, $P < 0.05$).

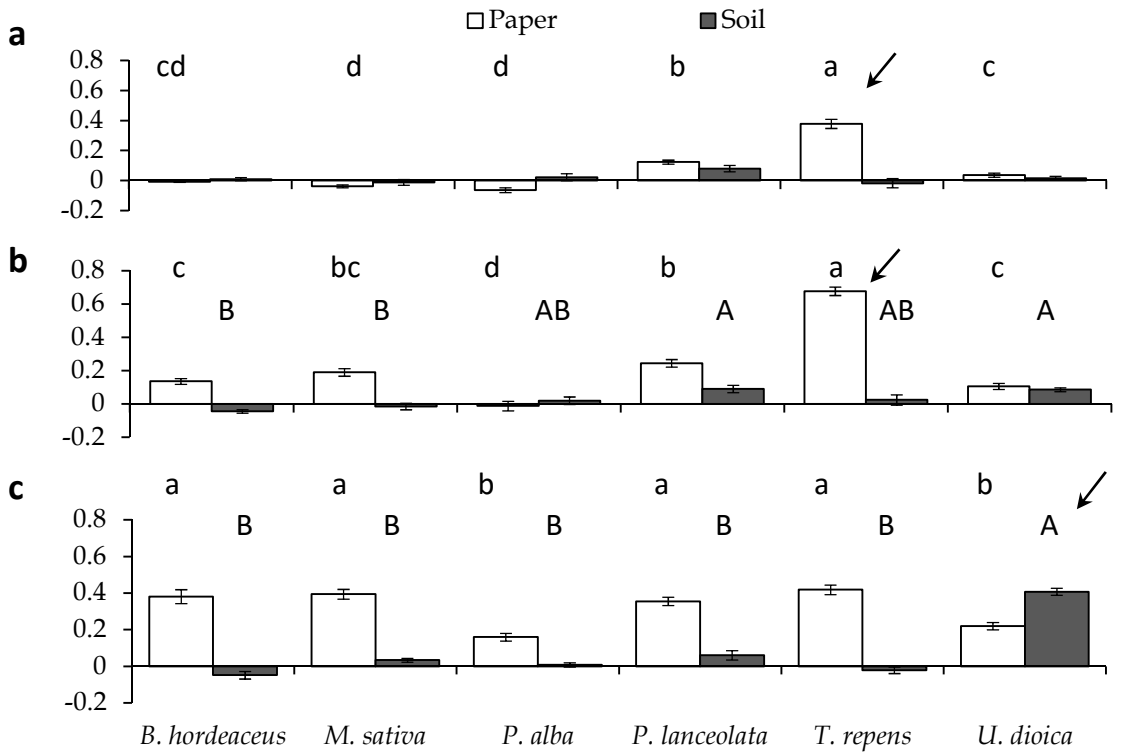
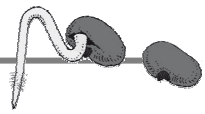


Figure 8. Second bioassay: Mean (±SE, n = 16) standardized percentage of seed germination (a), speed germination index (b) and radicle length (c) of the target species (*Bromus hordeaceus*, *Medicago sativa*, *Populus alba*, *Plantago lanceolata*, *Trifolium repens* and *Urtica dioica*) in paper (white bars) and soil (gray bars) submitted to leaf litter extracts from the different donor species (*Ailanthus altissima*, *Fraxinus angustifolia*, *Populus alba* and *Robinia pseudoacacia*) at different concentrations (0, 1, 2.5, 8.5 and 20 g L^{-1}). Different letters mean significant differences among target species in paper (lower case letters) and soil (uppercase letters) as substratum (Tukey HSD, $P < 0.05$). Arrows indicate the most sensitive species for each fitness indicator and substratum.

Discussion

Allelopathy has been considered as a plant-plant interaction mechanism structuring plant communities (Facelli and Picket 1991; Arroyo et al. 2015). Our study contemplates several donor and target species co-occurring in the same riparian area, which is relevant to interpret possible changes in species composition under the canopy of each donor tree species through allelopathy (Hierro and Callaway 2003; Inderjit and Nilsen 2003). In GP substratum, both native and EIPS reduced the fitness (G, SGI and the radicle growth) of several undercanopy species co-existing in riparian areas through leaf extracts at litter to water proportions that can be found in the field. Considering the complex allelopathic interaction among donor and target species we would expect different abundance of certain understory species in plots dominated by each tree species. However, multiple factors may finally determine the abundance and composition of the undercanopy plant communities. For example, the litter modification of the microclimatic and physical environment for seed germination (Facelli and Picket 1991; Skurski et al. 2014) or the secretion of substances by native plants that alleviates allelopathic effects (Weir et al. 2006). For that reason, the concordance between our experimental results and that of vegetation inventories in plots dominated by the different donor species could definitely confirm the displacement of certain undecanopy species by the EIPS throw allelopathy (Fernández et al. 2013; Arroyo et al. 2015; Da Silva et al. 2015), such as *T. repens*, *M. sativa* by *R. pseudoacacia* and *L. rigidum* by *A. altissima.* We also reported for the first time the allelopathic potential of the native species *F. angustifolia,* which was the only species reducing the SGI of *C. album* on GP substratum. Besides as far as we know this is the first time that has been documented the auto-allelopathy of the native *P. alba*, which is an important mechanism to regulate population density over space and time, avoid intra-competition, and to acquire better geographical distribution (Singh et al. 1999).

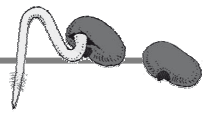


Plant chemistry is highly species-specific (Macel et al. 2014) and therefore specific compounds present in leaf extract of each donor species may selectively affect different target plants. The species-specific effects found in our study, mainly in GP substratum, indicate that the particular characteristics of each species may greatly determine its allelopathic potential more than its origin (exotic *vs*. native). For instance, specific allelopathic compounds have been identified in leaves of *A. altissima* (ailanthone, ailanthinone, chaparrine, and ailanthinol B; De Feo et al. 2003) and *R. pseudoacacia* (robinetin, myricetin, and quercetin; Nasir et al. 2005). Besides, the lowest allelopathic potential of *F. angustifolia* leaf extracts reducing radicle length of the target species could be explained by the reported lower concentration of total phenolic compounds in leaf litter from *F. angustifolia* than in that from the other donor species (Medina-Villar et al. 2015a). In addition, plant species containing large amounts of high molecular weight compounds (e.g. cellulose, lignin) usually have elevated quantities of secondary compounds in their tissues (Coley 1988). This could be the case of *R. pseudoacacia* and *P. alba* whose senescent leaves had higher concentration of lignin and acid detergent fiber than those of *F. angustifolia* and *A. altissima* (Medina-Villar et al 2015a) and were more effective against target species in our study. Even, lignin has been recognized as an allelochemical compound (Popa et al. 2008).

Studies including the comparison between native and EIPS allows to establish the relative importance of allelopathy for EIPS to affect native plant communities. In contrast to our expectations, our results did not support the NWH given that neither frequency nor the magnitude of the allelopathic effects produced by EIPS as a group were greater than those produced by native trees on undercanopy species. It indicates that the co-evolution between the undercanopy target species with the native trees, *F. angustifolia* and *P. alba,* was not sufficient reason for target species to better tolerate native than EIPS leaf extracts. Therefore, allelopathy appears as a general mechanism by which both native and EIPS affect other understory species.

We found different sensitivity among the target species to leaf extracts in GP substratum, being *A. stolonifera* (first bioassay) and *T. repens* (first and second bioassay) the most sensitive species to leaf extracts. Neither the taxonomic family, nor the life form or life spam could explain the different sensitivity of the target species. Nevertheless, specific seeds characteristics would explain these differences. For instance, seed size was proposed as an important factor affecting the sensitivity of the species to allelopathy (Olofsdotter et al. 1995; Kocacë and Terzi 2001), likely because of small seeds relay more on external resources and have further surface in permanent contact with the leaf extracts than large seeds. Thus, the lowest size of *A. stolonifera* seeds (http://data.kew.org/sid/) can explain their elevated sensitivity. However, experimental tests of the specific benefit of seed size for certain hazards are scarce (Westoby et al. 1992).

The high sensitivity of *T. repens* to allelopathy has also been reported by other authors (Singh et al. 1999; Catalán et al. 2013). In our study, *T. repens* was more sensitive than the other studied legume species, *M. sativa* and *Robinia pseudoacacia*. This could be explained by the smaller size and reserve concentration (oil and protein) of *T. repens* (http://data.kew.org/sid/). Fewer reserves in *T. repens* seeds imply that they rely more on external resources to germinate and grow, being more prone to be affected by the allelochemicals of the leaf extracts. Besides, since allelochemicals can effectively enter into the seed (Chiapusio et al. 2004) *T. repens* could be me more permeable to leaf extracts than the other target species. The seed permeability is related to seed coat characteristics, which greatly vary among species (Souza and Marcos-Filho 2001). For instance, among legumes, more pigmented seeds (brown color), such as *M. sativa* and *Robinia pseudoacacia* seeds (personal observation), have been reported to be more impermeable than less pigmented orange color seeds, such as *T. repens* seeds (personal observation) (Legesse and Powell 1996). Finally, we found fewer differences in radicle growth sensitivity among target species likely because radicle cells were more similar among species than seed coats.



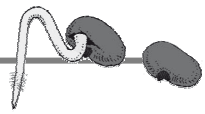
Over germination paper, radicle length and SGI were more responsive than G to the aqueous leaf extracts, according to other studies (Lawrence et al. 1991; Wardle et al. 1992; Catalán et al. 2013). This highlights the relevance of using different fitness indicators in allelopathy bioassays to better assess the sensitivity of different target species. More effects of leaf extracts on radicle growth than on G could be due to the germination success relies more on seed resources while radicle and plant growth rely more on external resources. The ecological consequence of reducing SGI and radicle growth may be the loss of optimum windows for establishment and growth (Verdú and Traveset 2005). The delay in germination of a species is disadvantageous to compete with other species for light (Miller et al. 1994). Even very short delays in seedling emergence have been reported to cause very negative effects on growth, biomass and reproduction of plant species living in forest undercanopies (Dyer et al. 2000; Verdu and Traveset 2005).

We found that the riparian soil clearly reduced the allelopathic effects observed in GP substratum. This buffer effect may be attributed to the sorption of allelochemicals by the soil organic matter (SOM) and/or to the degradation of these compounds by soil microorganisms (Kobayashi 2004; Pollock et al. 2009; Cipollini et al. 2012). In fact, riparian soils usually have high SOM and thus microbial activity (Schnürer et al. 1985; Naiman and Décamps 1997). The multiple interactions between allelochemicals and the soil environment make allelopathy difficult to be demonstrated in the field (Inderjit and Weiner 2001).

Although our study showed unlikely allelopathic effects under soil conditions, we did not discard the possibility that our study tree species affect undercanopy species under field conditions because of different reasons. Firstly, other studies documented allelopathic activity of *A. altissima* and *R. pseudoacacia* on other plant species in soil conditions (Lawrence et al. 1991; De Feo et al. 2003; Nasir et al. 2005; Gómez-Aparicio and Canham 2008a). The soils of these studies had different characteristics as the riparian soil we used regarding nutrients, SOM and texture, which are factors related to the

capability of soil to buffer allelopathic effects (Oleszek and Juzista 1987; Blum et al. 1993; Pollock et al. 2009). Secondly, the concentrations of dissolved allelopathic compounds in the soil could be greater considering plots with higher tree density than those used for our calculations or additional sources of allelopathic compounds released by plants, such as root exudates or lixiviates from other plant organs (Heisey 1990; Lawrence et al. 1991; De Feo et al. 2003; Kobayashi 2004) and cumulative effects of allelopathic compounds if they were slowly degraded (Sosa et al. 2010). However, real concentrations in the soil could be also lower due to rainfall likely extract fewer compounds than the soaking in the laboratory. Therefore, likely greater concentrations of allelopathic substances in the field would surpass the capability of soil to reduce allelopathy (Kobayashi 2004) but we unknew the real concentrations of allelopathic compounds in the soil.

U. dioica was the only species affected by the leaf extracts when soil was used as germination substratum. However it was affected almost equally by all donor species so that, presumably, it would not be affected by the invasion of *A. altissima* and *R. pseudoacacia*. Besides, *U. dioica* is a nitrophilous and shade tolerant species usually found under *R. pseudoacacia* canopies (Cierjaks et al. 2013), which were characterized by high soil nitrate concentration (Medina-Villar et al. 2016). Greater effects of the leaf extracts on radicle length of *U. dioica* in soil than in GP substratum may respond to the fact that soil microorganisms may transform chemical compounds with little phytotoxic activity into substances which may be more noxious for *U. dioica* (Kobayashi et al. 2004; Cipollini et al. 2012).



Conclusions

Our study showed that native and EIPS can potentially affect several co-existing undercanopy species by means of leaf extracts at realistic litter to water proportions. Leaf extracts of EIPS did not produce greater allelopathic effects than native species, against the NWH. Species-specific effects between donor and target species could be due to specific chemical composition of leaves from the donor species and may imply different plant composition under the canopy of the different tree species. Therefore, this study constitutes a first step to understand which understory species could be negatively affected by means of allelopathy in riparian forest invaded by the EIPSs. The riparian soil greatly reduced the allelopathic effects found in GP substratum, which questions the allelopathy under field conditions. Therefore, we greatly recommended the use of soil as substratum in future laboratory bioassays of allelopathy to avoid overestimation of allelopathic effects.

Acknowledgements

This study was supported by the projects CGL2011-16388/BOS and CGL2015-65346-R of the *Ministerio de Economía y Competitividad* of Spain, POII10-0179-4700 of the *Junta de Comunidades de Castilla-La Mancha* and *Ayuda Programa Grupos UCM-Banco Santander 2014 (Grupo 910314 Ecología Vegetal Evolutiva y Restauración Ecológica*). Silvia Medina Villar was supported by a grant from the *Ministerio de Economía y Competitividad* of Spain (FPI fellowship, BES-2011-048379). We are grateful to the support of the REMEDINAL3-CM MAE-2719 network (Comunidad de Madrid) and to Aitor Rodríguez Lopezosa for the help with the lab work.

Supplementary Information

Online resource 1. Mean (± SE, n = 10) pH and conductivity of deionized water (Control) and of the aqueous leaf litter extracts from the exotic (*Ailanthus altissima* and *Robinia pseudoacacia*) and native (*Fraxinus angustifolia*, *Populus alba*) donor tree species at different concentration of leaf litter per water volume.

	Donor tree species (Origin)	Concentration ($g\ L^{-1}$)	pH	Conductivity ($\mu S\ cm^{-1}$)
Control		0	7.17 ± 0.16	1.3 ± 0.2
Leaf extracts	*A. altissima* (exotic)	1	6.18 ± 0.14	107.4 ± 11.3
		2.5	6.13 ± 0.11	204.5 ± 26.5
		8.5	5.80 ± 0.09	706.9 ± 61.0
		20	5.72 ± 0.14	1507.4 ± 62.9
	F. angustifolia (native)	1	6.45 ± 0.13	124.0 ± 13.7
		2.5	6.46 ± 0.12	283.4 ± 27.6
		8.5	6.25 ± 0.16	753.6 ± 40.1
		20	6.22 ± 0.17	1563.4 ± 73.8
	P. alba (native)	1	6.29 ± 0.11	67.9 ± 8.7
		2.5	6.22 ± 0.10	145.0 ± 12.3
		8.5	6.02 ± 0.08	397.5 ± 28.1
		20	5.90 ± 0.10	906.0 ± 58.2
	R. pseudoacacia (exotic)	1	6.59 ± 0.11	113.6 ± 10.3
		2.5	6.38 ± 0.14	221.7 ± 11.5
		8.5	6.26 ± 0.13	658.6 ± 36.7
		20	6.24 ± 0.13	1393.6 ± 77.6

Online resource 2. Summary results of the three-way ANOVA assessing the effects of Substratum, Donor species , Concentration and their interactions on germination, speed germination index (SGI) and radicle length of the target species (*Trifolium repens*, *Medicago sativa*, *Populus alba*, *Bromus hordeaceus*, *Urtica dioica* and *Plantago lanceolata*)

	Target species											
	T. repens		*M. sativa*		*P. alba*		*B. hordeaceus*		*U. dioica*		*P. lanceolata*	
Germination (%)	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Substratum	313.03	***	0.64	0.43	16.25	***	2.44	0.12	100.25	***	19.31	***
Donor species	8.12	***	0.87	0.46	6.16	***	0.48	0.70	1.62	0.19	3.01	*
Concentration	57.54	***	1.91	0.11	1.04	0.39	2.31	.	1.41	0.23	13.00	***
susbstrate*donor species	5.48	***	1.06	0.37	3.12	*	0.44	0.72	4.63	***	1.48	0.22
substratum*concentration	52.09	***	0.60	0.66	2.15	0.08	0.78	0.54	1.37	0.25	3.55	***
donor species *concentration	1.51	0.13	0.49	0.92	0.63	0.81	0.45	0.94	1.77	.	1.86	*
susbstrate*donor species *concentration	1.40	0.17	1.19	0.30	0.81	0.64	0.94	0.51	0.97	0.48	1.39	0.18
SGI												
Substratum	155.36	***	1.24	0.27	91.90	***	161.33	***	205.36	***	17.35	***
Donor species	1.36	0.26	4.64	**	8.45	***	4.31	**	2.77	*	0.89	0.45
Concentration	38.56	***	22.37	***	3.83	**	22.52	***	6.14	***	15.17	***
susbstrate*donor species	2.08	0.11	3.54	*	3.76	*	0.09	0.97	2.60	.	3.02	*
substratum*concentration	25.72	***	12.41	***	2.49	*	26.86	***	0.80	0.53	5.66	***
donor species *concentration	1.54	0.11	0.89	0.56	1.33	0.21	2.30	*	2.04	*	2.86	***
susbstrate*donor species *concentration	1.52	0.12	1.32	0.21	0.91	0.54	1.27	0.24	0.82	0.63	1.03	0.42
Radicle length (mm)												
Substratum	38.70	***	509.41	***	361.24	***	28.38	***	13.35	***	257.73	***
Donor species	10.93	***	6.96	***	11.92	***	19.92	***	2.71	.	4.39	***
Concentration	69.14	***	124.52	***	21.80	***	48.94	***	59.63	***	30.50	***
susbstrate*donor species	8.24	***	15.93	***	2.18	.	33.87	***	1.37	0.26	10.64	***
substratum*concentration	73.02	***	107.92	***	18.72	***	39.94	***	7.62	***	17.63	***
donor species *concentration	2.00	*	1.97	*	1.61	0.11	4.22	***	0.94	0.51	2.29	*
susbstrate*donor species *concentration	2.47	***	2.57	***	2.29	*	4.75	***	1.02	0.44	2.63	***

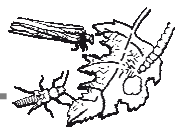
Signification codes: '***' < 0.001 ; '**' < 0.01; '*' < 0.05; '.' < 0.1

Capítulo 5

Decomposition and biological colonization of native and exotic leaf litter in a Central Spain stream

Lo que ahoga a alguien no es caerse al río, sino mantenerse sumergido en él.

Paulo Coelho



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This chapter reproduces the text of the following manuscript:

Medina Villar S, Alonso A, Vázquez de Aldana BR, Pérez-Corona E, Castro-Díez P (2015) Decomposition and biological colonization of native and exotic leaf litter in a Central Spain stream. Limnetica 34 (2): 293-310.

Fotografía: Arroyo Alboreca, cuenca alta del Río Henares (Guadalajara, España), mostrando las bolsas de descomposición de hojarasca usadas en este estudio.
Por: Silvia Medina Villar

Photography: Alboreca stream, upper basin of the Henares River (Guadalajara, Spain), showing the leaf litter bags used in this study.
By: Silvia Medina Villar

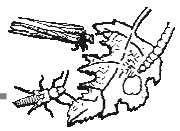
El dibujo de los encabezados está modificado de Allan y Castillo (2007)

The picture of headers is a modification from Allan and Castillo (2007)

Resumen

Descomposición y colonización biológica de hojarasca nativa y exótica en un arroyo del centro de España

La invasión de las riberas por árboles exóticos puede provocar cambios en la calidad de la hojarasca que entra en los ecosistemas de agua dulce, lo que es especialmente importante en arroyos de cabecera, donde las redes tróficas dependen en gran medida de los aportes de materia orgánica proporcionada por la vegetación terrestre. En un arroyo de cabecera del centro de España, Norte de la provincia de Guadalajara (Cuenca del Tajo), comparamos la descomposición y colonización biológica de la hojarasca de dos árboles exóticos (*Ailanthus altissima* (Mill.) Swingle y *Robinia pseudoacacia* L.) y dos nativos (*Fraxinus angustifolia* Vahl. y *Populus alba* L.), que coexisten en zonas de ribera. Se esperaba una menor colonización biológica de las hojas exóticas debido a que los organismos del arroyo no han co-evolucionado con los árboles exóticos de acuerdo con la Hipótesis de las Nuevas Armas (NWH) y, por tanto, menores tasas de descomposición de éstas. Para comprobar nuestra hipótesis, se sumergieron en el arroyo bolsas de descomposición con distinta luz de malla, gruesa (C) y fina (F) - usadas para distinguir entre la descomposición total y microbiana - y se recuperaron después de 2, 20, 39, 62 y 82 días de incubación. Después de cada recolección pesamos la cantidad de hojarasca remanente, analizamos el nitrógeno (N) y fósforo (P) de la hojarasca, identificamos los macroinvertebrados y cuantificamos la biomasa fúngica. De acuerdo con nuestra hipótesis, encontramos mayor biomasa fúngica acumulada en hojas nativas que en exóticas. Sin embargo, la tasa de descomposición (k) fue específica de cada especie, siendo el ranking en las bolsas C (*A. altissima* = *F. angustifolia* > *P. alba* > *R. pseudoacacia*) ligeramente diferente al de las bolsas F (*A. altissima* > *F. angustifolia* > *P. alba* > *R. pseudoacacia*). Ambas k (en bolsas C y F) se correlacionaron con la lignina, la fibra ácido detergente (ADF) y el ratio lignina:P. Nuestros resultados indican que no hubo un efecto claro de las



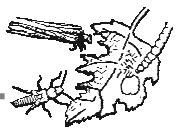
especies exóticas sobre la comunidad de macroinvertebrados, pero la contribución de los macroinvertebrados a la descomposición fue menor en la hojarasca exótica que en la nativa, como muestra el menor ratio entre k en bolsas C y F (k_C:k_F). Nuestros resultados apoyan que las especies invasoras pueden impactar tanto la colonización biológica como las tasas de descomposición de la hojarasca en los ecosistemas de arroyos a través del aporte de hojarasca de distinta calidad respecto de la vegetación nativa. Las diferencias en colonización biológica pueden ser explicadas por la NHW pero las diferencias en descomposición son mejor explicadas por la calidad de la hojarasca.

Palabras clave: *Ailanthus altissima*; biomasa fúngica; calidad de la hoja; comunidad de macroinvertebrados; ecosistemas de agua dulce; especies invasoras; *Robinia pseudoacacia*.

Abstract

Decomposition and biological colonization of native and exotic leaf litter in a Central Spain stream

Riparian invasion by exotic trees may lead to changes in the quality of leaf-litter inputs to freshwater ecosystems, especially important in headwater streams, where aquatic food webs largely depend on the organic matter provided by the terrestrial vegetation. In a headwater stream of Central Spain, North of Guadalajara Province (Tagus basin) we compared decomposition and biological colonization of leaf litter among two exotic (*Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L.) and two native trees (*Fraxinus angustifolia* Vahl. and *Populus alba* L.) co-existing in riparian zones. We hypothesized a lower biological colonization of exotic leaves due to organisms have not co-evolved with the exotic trees according to the *Novel Weapons Hypothesis* (NWH) and, consequently, a lower decomposition rates of exotic leaves. To test our hypothesis, litter bags with different mesh size, coarse (C) and fine (F) - used to distinguish between total and microbial decomposition - were placed in the stream and recovered after 2, 20, 39, 62 and 82 days of incubation. After each collection, we assessed the amount of remaining litter, analyzed the nitrogen (N) and phosphorous (P) in the litter, identified the macroinvertebrates and quantified the fungal biomass. Consistent with our hypothesis, we found greater fungal biomass buildup on native than on exotic leaves. However, decomposition rates (k) were species-specific, being the ranking in C-bags (*A. altissima* = *F. angustifolia* > *P. alba* > *R. pseudoacacia*) slightly different than in F-bags (*A. altissima* > *F. angustifolia* > *P. alba* > *R. pseudoacacia*). Both k (in C and in F-bags) were correlated with leaf traits such as, lignin, acid detergent fiber (ADF) and lignin:P. There were not a clear effect of exotic species on macroinvertebrate variables, but the contribution of macroinvertebrates to litter decomposition was lower in exotic than in native litter, as shown by a lower ratio between k in C and k in F bags (k_C:k_F). Our results support that invasive species might impact both biological colonization and decomposition rates in



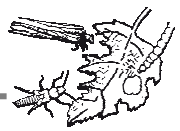
stream ecosystems by the addition of different quality leaf litter as compared to native vegetation. Differences in biological colonization may be explained by the NWH but differences in decomposition rates are better explained by litter quality.

Keywords*: Ailanthus altissima*; freshwater ecosystem; fungal biomass; invasive species; leaf quality; macroinvertebrate community*; Robinia pseudoacacia.*

Introduction

Litter inputs into streams from riparian areas represent the main food source for heterotrophic communities in headwater streams, where the shade cast by riparian canopies limits autochthonous primary production (Wallace et al. 1997). In temperate deciduous forest, fallen leaves during autumn are the main component of the litter inputs (Abelho 2001). When leaf litter falls into a stream a complex decomposition process begins, which involves an initial leaching of soluble compounds, followed by physical abrasion and colonization by microbes and invertebrates (Webster and Benfield 1986). The rate of leaf decomposition depends on the local climatic conditions, water properties, biotic communities, as well as on the quality of the leaves (Webster and Benfield 1986). Leaf chemical composition (e.g. content of nitrogen (N) phosphorous (P), phenolic compounds, and lignin) along with physical properties (e.g. leaf toughness, leaf mass per unit of area, and density) represent the leaf quality, which is a major factor determining decomposability of leaves (Enriquez et al. 1993; Ostrofsky 1997; Graça and Canhoto 2006; Bottollier-Curtet et al. 2011).

The quality of leaf litter may affect aquatic detritivores. For instance, leaf secondary compounds, such as polyphenols or essential oils, may delay or inhibit fungal growth (Graça et al. 2002; Mathuriau and Chauvet 2002), which in turn, may affect leaf consumption by macroinvertebrates since microbial colonization (i.e. conditioning) make leaves more palatable to detritivores (Arsuffi and Suberkropp 1989; Wright and Covich 2005). Besides, it was reported that aquatic macroinvertebrates preferentially consume soft leaves with high N content and low amounts of structural carbon (Motomori et al. 2001; Rincón and Martínez 2006). The growth and survival of macroinvertebrates increase with the consumption of such high-quality leaves (Albariño and Balseiro 2002; Going and Dudley 2008). Density and diversity of invertebrates may be directly affected by exotic trees bearing leaves with high secondary compounds, as is the case of *Eucalyptus* spp. (Larrañaga et al. 2009; Gama et al. 2014). However, in the *Eucalyptus* spp. native range, the diversity of



invertebrates is reduced by the introduction of the exotic *Salix* spp. (Read and Barmuta 1999). The negative effect of eucalyptus on invertebrates in its exotic but not in its native range suggests that invertebrates in the native range are adapted to chemicals present in *Eucalyptus* spp. leaves, which are novel for those in the exotic range. In this context, the *Novel Weapons Hypothesis* described by Callaway and Ridenour (2004) predicts that secondary chemical compounds produced by exotic species affect microorganisms and invertebrates in their areas of introduction, because these organisms are not adapted to these compounds.

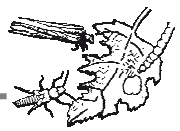
Riparian ecosystems are highly vulnerable to exotic plant invasion (Hood and Naiman 2000) due to their milder microclimatic conditions and the frequent natural and anthropogenic disturbances which create diverse niches to support invasive species (Planty-Tabacchi et al. 1996; Chytrý et al. 2008). Vegetation destruction by human activities additionally increases the invasibility of these systems, creating gaps that can be readily colonized by light-demanding invaders (Lindig-Cisneros and Zedler 2001). Floodplain invasions by exotic trees may alter the quality of leaf litter inputs into streams, thus causing effects on heterotrophic communities and nutrient cycles (Bailey et al. 2001; Hladyz et al. 2009). Those effects will depend on the magnitude and direction of the leaf quality differences between the exotic and the native species (Hladyz et al. 2009).

Ailanthus altissima (Mill.) Swingle (Simaroubaceae) and *Robinia pseudoacacia* L. (Fabaceae) are two aggressive invasive tree species in many regions of the world and they usually invade riparian areas (Sanz-Elorza et al. 2004; GEIB 2006; DAISIE 2009). *A. altissima* is native to Southeast Asia and *R. pseudoacacia* is native to East and Central United States (Kowarik and Säumel 2007; Cierjacks et al. 2013). Some of the traits that may contribute to their invasive potential are the production of phytotoxic compounds (De Feo et al. 2003; Nasir et al. 2005), a profuse resprouting capacity and, in the case of *R. pseudoacacia*, the ability to fix atmospheric N_2, which is very useful in nutrient-

poor soils (Rice et al. 2004; Kowarik and Säumel, 2007; Cierjacks et al. 2013). Both exotics are in the Atlas of exotic invasive plants in Spain (Sanz-Elorza et al. 2004) and they co-occur with native riparian trees, such as *Fraxinus angustifolia* Vahl. (Oleaceae) and *Populus alba* L. (Salicaceae).

Some studies have investigated the effect of *A. altissima* and *R. pseudoacacia* on riparian soils and on lentic ecosystems of Central Spain (Alonso et al. 2010; Castro-Díez et al. 2012), but studies on stream ecosystems which are highly depend on terrestrial inputs from riparian vegetation are scarce (but see Swan et al. 2008). Moreover, studies about the effect of *A. altissima* and *R. pseudoacacia* on fungal colonization of leaves are lacking even when aquatic fungi are an important component involved in leaf decomposition (Abelho 2001; Cornut et al. 2010).

The aim of this study was to assess the impacts of *A. altissima* and *R. pseudoacacia* on key functions of a stream ecosystem in Central Spain. Specifically, we compared leaf decomposition rates, fungal and macroinvertebrate colonization, and nutrient dynamics between exotic and two common native tree species (*F. angustifolia* and *P. alba*). Since leaf quality is the main factor determining leaf decomposition through the effect on macroinvertebrates and fungi, differences in some leaf traits among species were also explored. We hypothesized that exotic leaf litter will show lower fungal and macroinvertebrate colonization than native leaf litter given that stream organisms have not co-evolved with the exotic tree secondary compounds (*Novel Weapons Hypothesis*; Callaway and Ridenour 2004) and, consequently, decomposition rates of the exotic leaf litter in the stream are expected to be lower than those of the native leaf litter.



Materials and Methods

Study area and leaf collection

The experiment was performed in the Alboreca stream, in the upper basin of the Henares River (Guadalajara, Spain; latitude 41 º 8 ´ N, longitude 2 º 36 ´ W, elevation 1056 m). The climate in the area is mediterranean pluviseasonal-oceanic with mean maximum and minimum annual temperature of 31.5 ºC and –2.1 ºC, respectively, and the mean annual precipitation of 601 mm (Worldwide Bioclimatic Classification System, http://www.globalbioclimatics.org). The riparian vegetation of the upper basin of Henares River is characterized by native trees *P. alba and F. angustifolia* with some patches invaded by the exotic tree species *A. altissima* and *R. pseudoacacia*. At the study site, the floodplain is mostly occupied by crops and *Populus x euramericana* plantations (**Anexo, foto 5**).

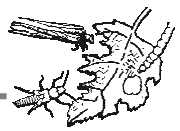
The wetted width, stream depth and current velocity of the study site were measured at the beginning and the end of the experiment using a meter stick and a portable velocimeter. Water maximum and minimum temperature, pH, conductivity and nutrient concentrations were monitored during the leaf litter decomposition experiment at each collection date. The water temperature was measured with an outdoor maximum/minimum thermometer placed inside the stream. In the laboratory, the stream water pH and conductivity were measured with a pH meter (Crison micropH 2001) and a conductivity meter (Crison conductivity meter 524), respectively, and the water nitrate, ammonium and ortho-phosphate were measured using photometric methods (Spectroquant® test).

Senescent leaves, i.e. those which detach with a gentle touch, from *R. pseudoacacia*, *A. altissima*, *F. angustifolia* and *P. alba* were collected in October 2009 from the riparian forest along the low stretch of the Henares River (near the city of Alcalá de Henares, Madrid, Spain). For each species, leaves were collected from multiple trees, pooled and air-dried at room temperature.

Rachises from compound leaves (*A. altissima*, *F. angustifolia* and *R. pseudoacacia*) and petioles of *P. alba* were removed in order to homogenize the leaf sampling used for this experiment. Although we only used leaf laminas, we will refer them as "leaves" throughout the paper.

Leaf litter decomposition

Air-dried leaves from each tree species were incubated in the stream using bags with two different mesh size: 1) fine mesh bags (F-bags) with 12 × 12 cm were made with 1.2 mm nylon mesh size, which excluded macroinvertebrate access and allowed only microbial decomposition; 2) coarse mesh bags (C-bags) with 17 × 17 cm were made with high density 5 mm polyethylene mesh size and allowed both microbial and macroinvertebrate processing (**Anexo, foto 6**). Each bag was filled with ca. 2 g of senescent air-dried leaves of a single species. In 12 November 2010, a total of 252 litter bags were placed in two stream riffles (**Anexo, foto 7**), separated 15 m from each other. Among them, 160 litter bags (4 leaf species × 2 types of litter bags × 5 collections × 4 replicates) were used to assess litter decomposition and to identify the macroinvertebrates colonizing litter material. Besides, 72 additional litter bags (4 leaf species × 2 types of litter bags × 3 collections × 3 replicates) were used for fungal biomass estimation (see below). The remaining 20 bags were C empty bags (5 collections × 4 replicates) that were used as a control for the macroinvertebrates that used the bags as refuge. Each replicated bag (one per species, litter bag type and collection) was fixed in a random position to one of two chains, fastened to one of four concrete blocks (**Anexo, foto 6**). Half of these blocks were placed in each riffle (**Anexo, foto 7**). In this way, the potential effect of the riffle and the block on litter decomposition and colonization was homogeneously distributed among species, bag types and collections. Litter bags were collected after 2, 20, 39, 62, and 82 days of incubation by means of a hand net. Previous to the third collection there was a sudden rise of the water flow (detected 40 km downstream 12 days before the 3rd collection by Bujalaro gauging station), which moved all concrete blocks from their initial position,



and possibly dragged part of the invertebrates which had already colonized the litter bags. However no litter bag or its content was lost. Three days before the third collection blocks were placed back to their initial position, to keep the initial site conditions. After each collection, samples were stored in polyethylene zipper bags, transported and kept cold (5 ^{o}C) until processing. Once in the laboratory, litter bags were gently rinsed with tap water over a 250 µm sieve to separate macroinvertebrates from leaves. Macroinvertebrates were preserved in a 3 % formaldehyde solution until identified to family level using Tachet et al. (2003). Macroinvertebrates were additionally classified into the following functional feeding groups (FFG): shredders (SH), collector-gatherers (CG), collector-filterers (CF), scrapers (SC) and predators (PD), according to Merritt and Cummins (1996) and Tachet et al. (2003). Leaf mass remaining (LMR) in each bag was oven-dried (≥ 48 h at 60 ^{o}C) and weighed.

Leaf traits

We assessed N and P concentrations in 1) three 2 g-replicates of the initial senescent leaves and 2) the total remaining leaf mass in each collection and species from C-bags. Samples were oven-dried (≥ 48 h at 60 ^{o}C) to correct for water content and ground with a Culatti mill to a particle size < 0.5 mm. N and P concentrations were determined with a segmented flux auto-analyzer (Skalar San^{++}), after a digestion with H_2SO_4 and Cu−KSO_4 (Nelson and Sommers 1973). Acid detergent fiber (ADF), lignin and total phenolic compounds (TPC) were analyzed only in non-decomposed leaves. ADF and lignin concentrations were determined using the filter bag technique (FBT), with an Ankom Automated Fiber Analyzer A2000, based on the analytical method of Goering and Van Soest (1970). The extraction procedure to determine TPC was performed in duplicate as follows: 200 mg of freeze-dried and ground leaves were extracted twice in 5.0 ml of 50:50 (v/v) methanol:water for 30 min in an ultrasound bath. The mixture was centrifuged and filtered twice through filter paper, and the filtrates were mixed and stored at –20 ^{o}C until analysis (Waterman and Mole 1994). The TPC concentration was assessed by the

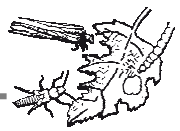
colorimetric Prussian-blue method using gallic acid as standard (Graham 1992). We calculated litter quality indexes N:P, lignin:N and lignin:P ratios since they have been previously related to decomposition rates and dynamics (Hladyz et al. 2009).

Fungal biomass determination

Fungal biomass in litter samples, collected after 20, 39 and 62 days in the stream, was estimated from the concentration of ergosterol, a specific component of fungal cell membranes (Ekblad et al. 1998), following the methodology of Gessner (2005). In brief, freeze-dried leaf samples (ca. 0.10 g) were placed in tubes with 10 ml of 0.14 M KOH in methanol. The mixture was incubated at 80 ^{o}C during 30 min in a Block Heater (Stuart Scientific). After being cooled, the extract was acidified with 5 ml of 0.75 M HCl and transferred into a SPE cartridge (Waters Sep-Pack Vac RC, 500 mg, tC18) pre-conditioned with an acidified extract solution (0.14 M KOH in methanol: 0.75 M HCl; 6:1 v/v). After sample addition, the column was washed with 2.5 ml of 0.4 M KOH in 60 % methanol (v/v) and dried under a stream of air for about 60 min. Ergosterol was eluted into HPLC vials with 1.6 ml of isopropanol. Final separation of ergosterol from matrix lipids was performed by HPLC with a Waters 2690 system with a Sunfire C18 Waters column (5 µm; 5.6 × 250 mm), at 33 ^{o}C. The mobile phase was methanol (100 %) at a flow rate of 1.4 ml min^{-1}. Ergosterol was detected at 282 nm with a Photodiode Array Detector (PDA) Waters 2996. Fungal biomass concentration was estimated using the conversion factor of 5.5 mg ergosterol per gram of fungal dry mass (Gessner and Chauvet 1993).

Statistical analysis

Analysis of covariance (ANCOVA) was performed, using time as a covariate, to assess the effect of the two factors (litter bag and species) on the percentage of leaf mass remaining (% LMR), as a dependent variable. The %

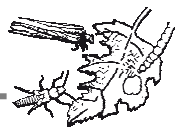


LMR was transformed ($Ln\,(x+1)$) to meet the linearity assumption for ANCOVA and it was fitted with time to calculate the decomposition rates (k) in C and F-bags (k_C and k_F, respectively). We considered that two species differed in k when their 95 % confident intervals did not overlap. The ratio k_C:k_F was considered as a proxy measure of the effect of macroinvertebrates on leaf litter decomposition, although physical abrasion by water flow is also a factor which affect litter decomposition and act in greater extent in C than in F-bags (Hladyz et al. 2009). Therefore, changes in the k_C:k_F ratio across species implies shifts in the relative contribution of microorganisms and shredders to litter decomposition (Gessner and Chauvet 2002). We did not used the last collection (82 days) in the analysis because at this collection no remains of *A. altissima* and *F. angustifolia* leaves were found and increments in % LMR were noticed in *R. pseudoacacia* and *P.alba,* likely due to $CaCO_3$ precipitation (Casas and Gessner 1999). Differences among species in initial leaf traits were assessed by one-way ANOVA followed by Tukey HSD test. To meet ANOVA assumptions P concentration was Ln transformed. Pearson correlations between leaf traits and k were performed to determine which traits control leaf decomposition. Differences in N and P concentrations of decomposed leaves among species and time were assessed by two-way ANOVA (species × time) followed by Tukey HSD test. Differences in fungal biomass were assessed by three-way ANOVA (species × litter bag × time) followed by Tukey HSD test.

Differences in total density (number of individuals per litter bag) and richness (number of macroinvertebrate families per litter bag) were assessed using two-way ANOVA (species × time). To meet ANOVA assumptions, the density of macroinvertebrate families were log-transformed. A generalized linear model (GLM, Poisson distribution, log link) was used to evaluate differences in the relative abundance of shredders across species and time. These analyzes included the control (empty bags) as a level of the factor species.

To search for patterns among the macroinvertebrate communities colonizing the different leaf species, a two-dimensional non metric

multidimensional scaling ordination (NMDS) (McCune and Grace 2002), was performed on the mean relative abundance of macroinvertebrate families (number of individuals of a family per total number of individuals in litter bag). Macroinvertebrate families that occurred in less than 5 % of the C-bags (i.e. 26 out of 42 families) were omitted to reduce the effect of rare taxa on the ordination (McCune and Grace 2002). Mean relative abundance of the used families per litter species and collection is available in Supplementary information (Table S1 at www.limnetica.net/internet). The sample positions on the NMDS biplot were calculated by Euclidean distances from the original data. Fifteen iterations were run to achieve a stress value of 0.15, which is considered satisfactory in Community Ecology (McCune and Grace 2002). Pearson correlations between mean relative abundance of each macroinvertebrate family and each axis of NMDs were performed to assess which macroinvertebrate families had further weight on the ordination. Families with $R^2 > 0.6$ and a significant correlation ($p < 0.05$) were considered as the most influential on the ordination. Permutational multivariate analysis of variance (perMANOVA) using Bray-Curtis distance matrices were applied to the relative abundance of macroinvertebrate families used in the NMDS to assess differences across species and time in the macroinvertebrate community structure. R package 2.14.0 (R Development Core Team, 2011) was used for NMDS ordination (isoMDS algorithm in R) and perMANOVA (Adonis algorithm in R) analyzes, while JMP, Version 7 (SAS Institute Inc., Cary, NC, 1989-2007), was used for the rest of analyzes.



Results

Stream characteristics

The mean ± SE properties of the study stream were as follows: stream wetted width = 2.23 ± 0.11 m (n = 21), depth = 16 ± 1 cm (n = 157), current velocity = 0.25 ± 0.02 m s^{-1} (n = 157), water conductivity = 607.4 ± 18.9 µs cm^{-1} (n = 5), pH = 8.05 ± 0.04 (n = 5), water minimum – maximum temperature = 5 - 13 °C and nutrient concentrations = 2.9 ± 0.5 mg NO_3−N l^{-1}, 0.17 ± 0.03 mg NH_4−N l^{-1}, and 0.06 ± 0.01 mg PO_4−P l^{-1} (n = 5). Therefore, our stream was P limited (N:P > 40).

Leaf traits

A. altissima leaves showed relatively low lignin and ADF concentration and high N, P and TPC concentrations (Table 1). By contrast, *R. pseudoacacia* leaves showed relatively low P, the highest N and relatively high concentrations of lignin, ADF and TPC. *F. angustifolia* leaves showed similar properties to *A. altissima* except for the lower N and TPC. *P. alba* leaves exhibited high lignin, ADF and TPC concentrations and low N and P.

Table 1. Mean values (±SE, n = 3) for initial leaf traits of exotic and native species. N = Nitrogen; P = Phosphorus; TPC = Total phenolic compounds; ADF = Acid Detergent Fiber. Different letters in a row indicate significant differences among species for each variable (ANOVA test with Tukey HSD, $p < 0.05$) and they are alphabetically ordered from the higher to the lower value.

	Exotics		*Natives*	
Leaf traits	*A. altissima*	*R. pseudoacacia*	*P. alba*	*F. angustifolia*
N (%)	1.51 ± 0.01 ^{b}	1.75 ± 0.01 ^{a}	0.93 ± 0.02 ^{d}	1.00 ± 0.00 ^{c}
P (%)	0.10 ± 0.01 $^{a\,b}$	0.07 ± 0.00 ^{c}	0.07 ± 0.00 $^{b\,c}$	0.15 ± 0.02 ^{a}
Lignin (%)	18.1 ± 0.7 ^{b}	29.5 ± 1.5 ^{a}	32.6 ± 1.8 ^{a}	22.2 ± 0.5 ^{b}
ADF (%)	23.1 ± 1.3 ^{c}	31.7 ± 0.3 ^{b}	36.1 ± 1.1 ^{a}	26.0 ± 0.6 ^{c}
TPC (%)	2.3 ± 0.4 ^{a}	2.7 ± 0.0 ^{a}	2.2 ± 0.1 ^{a}	1.1 ± 0.0 ^{b}
N:P	14.9 ± 1.5 ^{b}	26.3 ± 0.8 ^{a}	13.2 ± 0.5^{b}	6.8 ± 0.9 ^{c}
Lignin:N	11.9 ± 0.4 ^{c}	16.9 ± 0.9 $^{b,\,c}$	35.2 ± 2.3 ^{a}	22.2 ± 0.6 ^{b}
Lignin:P	178. 9 ± 23.8 ^{b}	444.9 ± 34.9 ^{a}	465.5 ± 41.4^{a}	149.8 ± 16.4 ^{b}

Leaf litter decomposition

The ranking of litter species by k_C (in presence of macroinvertebrates) was: *A. altissima* = *F. angustifolia* > *P. alba* > *R. pseudoacacia*, while by k_F (in absence of macroinvertebrates) was: *A. altissima* > *F. angustifolia* > *P. alba* > *R. pseudoacacia* (Table 2; Fig. 1a-b). All species, except *A. altissima*, showed significantly greater k_C than k_F (Table 2). The interaction terms (species × time) and (litter bag × time) were highly significant (ANCOVA; $F_{3,121} = 49.4$, and $F_{1,123} = 56.1$, respectively; $p < 0.0001$) indicating that both factors (species and litter bag) had a significant effect on decomposition rates (Figs. 1a-b). In addition, the interaction term (species × litter bag × time) was also significant (ANCOVA, $F_{3,124} = 3.67$, $p = 0.014$) indicating that the effect of litter bag in decomposition rates was different among species. The contribution of macroinvertebrates to decomposition (ratio k_C:k_F) was greater in native than in exotic leaf litters (Table 2). The k_C was negatively correlated with lignin (%), ADF (%), N:P ratio and lignin:P and positively with P (%) (Pearson correlation; $r = -0.82$, $r = -0.77$, $r = -0.73$, $r = -0.86$ and $r = 0.66$, respectively; $p < 0.05$) while the k_F was negatively correlated with lignin (%), ADF (%) and lignin:P (Pearson correlation; $r = -0.86$, $r = -0.81$ and $r = -0.75$, respectively; $p < 0.05$), but no significant correlations were found between decomposition rates and the rest of leaf traits considered (N, TPC, and lignin:N).

Table 2. Decomposition rates (k) (Mean ± 95% Confidence interval) in coarse (k_C) and fine (k_F) litter bags, the k_C:k_F ratio of the four species and the R^2 coefficient of each regression line fitted to calculate k. Different letters in a column indicate significant differences among species ($p < 0.05$, overlap test). For each species, significant differences between k_C and k_F ($p < 0.05$, overlap test) are indicated by an asterisk.

	Decomposition rate				
Species	$-k_C$ (Mean (95 % CI))	R^2	$-k_F$ (Mean (95 % CI))	R^2	k_C:k_F
A. altissima	0.080 (0.062-0.098) [a]	0.87	0.053 (0.040-0.066) [a]	0.84	1.51
R. pseudoacacia *	0.017 (0.012-0.022) [c]	0.80	0.008 (0.007-0.009) [d]	0.95	2.07
P. alba *	0.040 (0.029-0.051) [b]	0.82	0.016 (0.012-0.020) [c]	0.84	2.50
F. angustifolia *	0.069 (0.058-0.081) [a]	0.93	0.028 (0.022-0.034) [b]	0.89	2.43

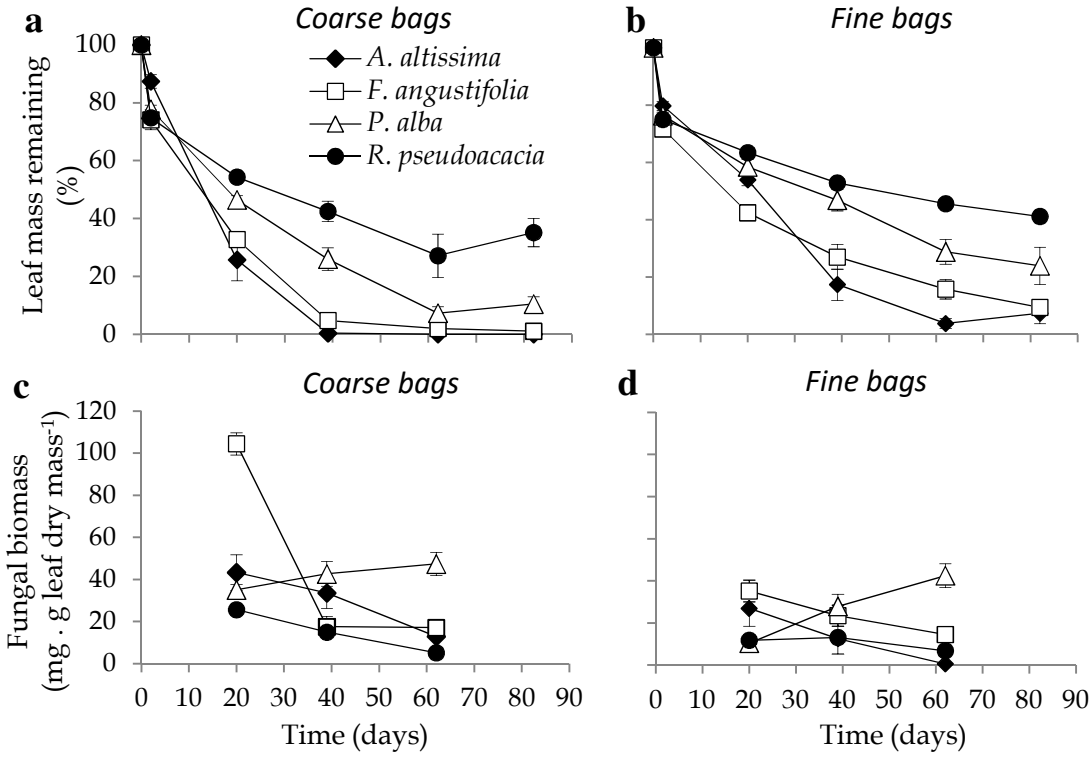


Figure 1. Mean (± SE) leaf mass remaining (n = 4) (a and b) and fungal biomass (n = 3) (c and d) in the coarse (a and c) and fine (b and d) litter bags for each species over the study period. Black symbols indicate exotic species and white symbols native species.

Fungal biomass

Fungal biomass buildup on decomposing leaves was affected by species, time, litter bag (C and F) and most of the interactions (Table 3). Native leaf litter had higher fungal biomass than exotic leaf litter (ANOVA, Tukey HSD, $p < 0.05$; Figs. 1c-d). In the presence of macroinvertebrates (C-bags) differences in fungal biomass between exotic and native leaves were mainly due to the greater value in the native *F. angustifolia* at 20 days and the greater value in *P. alba* than in *R. pseudoacacia* at 62 days (ANOVA, Tukey HSD, $p < 0.05$; Fig. 1c). However, in the absence of macroinvertebrates (F-bags) both exotics showed lower fungal biomass than natives at 39 and 62 days (ANOVA, Tukey HSD, $p < 0.05$; Figs. 1d). In F-bags fungal biomass was lower than in C-bags but only significant in the case of *F. angustifolia* (ANOVA, Tukey HSD, $p < 0.05$; Figs. 1c-d). Fungal biomass did not vary significantly throughout the study for the two exotic

species in any bag type (C and F) (ANOVA, Tukey HSD, $p > 0.05$; Figs. 1c-d) but peaks were observed for the two natives at 20 days (*F. angustifolia*) and at 62 days (*P. alba*) (ANOVA, Tukey HSD, $p < 0.05$; Figs. 1c-d).

Table 3. Results of two-way ANOVA (species and time) for nitrogen (N) and phosphorous (P) concentration and three-way ANOVA (species, time and litter bag) for fungal biomass.

Source of variability	df	F	*p*
N (%)			
Species	3	84.8	**< 0.0001**
Time	4	72.7	**< 0.0001**
Species x time	12	9.2	**< 0.0001**
P (%)			
Species	3	76.7	**< 0.0001**
Time	4	11.4	**< 0.0001**
Species x time	12	4.6	**< 0.0001**
Fungal biomass (mg. g dry mass^{-1})			
Species	3	20.2	**< 0.0001**
Time	2	19.2	**< 0.0001**
Litter bag	1	33.5	**< 0.0001**
Species x time	6	17.3	**< 0.0001**
Species x litter bag	3	2.3	0.0922
Time x litter bag	2	2.7	0.0711
Species x time x litter bag	6	4.7	**0.0007**

Significant P-values are indicated in bold.

Nutrient dynamics

Litter N and P concentrations were influenced by species, time and the interaction between them (Table 3). Leaves of *R. pseudoacacia* showed the highest N and lowest P concentrations throughout the experiment (ANOVA, Tukey HSD, $p < 0.05$; Figs. 2a-b). N concentration increased in the second collection (20 days) in all species and then decreased in *A. altissima* and *F. angustifolia*, kept increasing in *P. alba* and remained unchanged in *R. pseudoacacia* (ANOVA, Tukey HSD, $p < 0.05$; Fig. 2a). In the first collection (day 2), P concentration decreased and then increased over time in *P. alba* leaves but no variation was observed for the other leaf species (ANOVA, Tukey HSD, Fig. 2b).

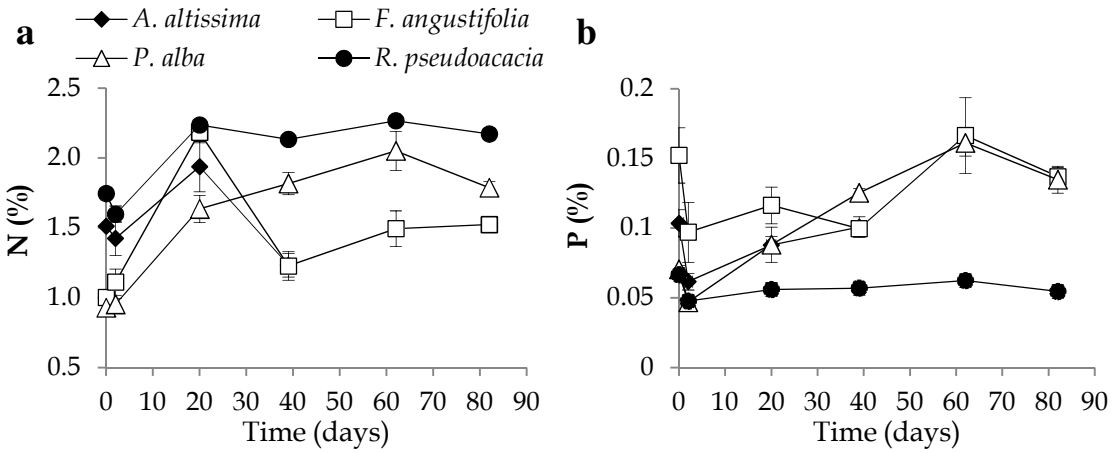


Figure 2. Mean (± SE, n = 4) leaf N (a) and P (b) concentrations for each species over the study period. Black symbols indicate exotic species and white symbols native species.

Macroinvertebrate colonization

The most abundant macroinvertebrate families along the study in the NE bags were: Chironomidae (Diptera), Gammaridae (Amphipoda), Bithyniidae (Mollusca), Hydropsychidae (Trichoptera), Baetidae (Ephemeroptera) and Simuliidae (Diptera) (Some of them showed in **Anexo, foto 8**) that made up 28 %, 23 %, 12 %, 10 %, 8 % and 5 %, respectively, of the total macroinvertebrate abundance. Total macroinvertebrate density and family richness did not vary across species (ANOVA, $F_{4, 84} = 1.75$, $F_{4,84} = 1.61$, respectively, $p > 0.05$) or time ($F_{4, 84} = 1.52$, $F_{4,84} = 2.04$, respectively, $p > 0.05$) but were affected by its interaction ($F_{16, 84} = 4.67$, $F_{16,84} = 3.07$, respectively $p < 0.05$; Figs. 3a-b). Therefore, the density and richness of the macroinvertebrate families varied in a different way along time. Macroinvertebrate density drastically dropped in the third collection (Fig. 3a) likely due to the sudden rise of flow mentioned above, which may have dragged many of the macroinvertebrates that had already colonized the litter bags. Subsequently, macroinvertebrate density increased in the leaf litters with faster decomposition (*A. altissima* and *F. angustifolia)*, but decreased in those with slower decomposition (*P. alba* and *R. pseudoacacia*) (Fig. 3a). A similar pattern appeared in family richness, which increased in *A. altissima* and *F. angustifolia* and decreased in *P. alba* and *R. pseudoacacia* from 39 to 62 days (Fig. 3b). However, the relative abundance of shredders did not

differ across species, time or its interaction (GLM, Chi^2 = 1.12, Chi^2= 1.39, Chi^2= 3.85, respectively, $p > 0.05$).

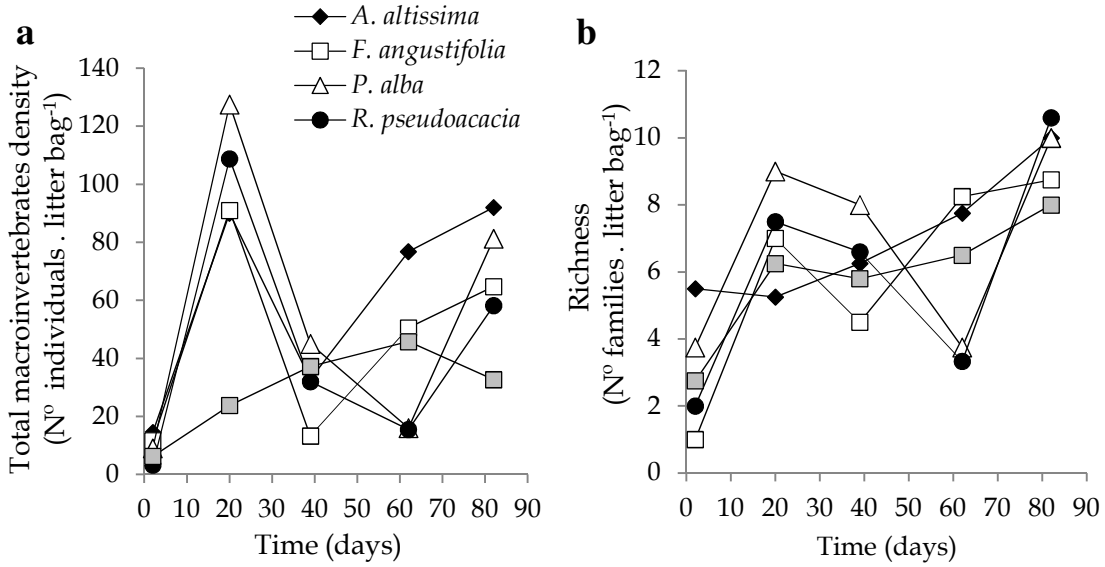
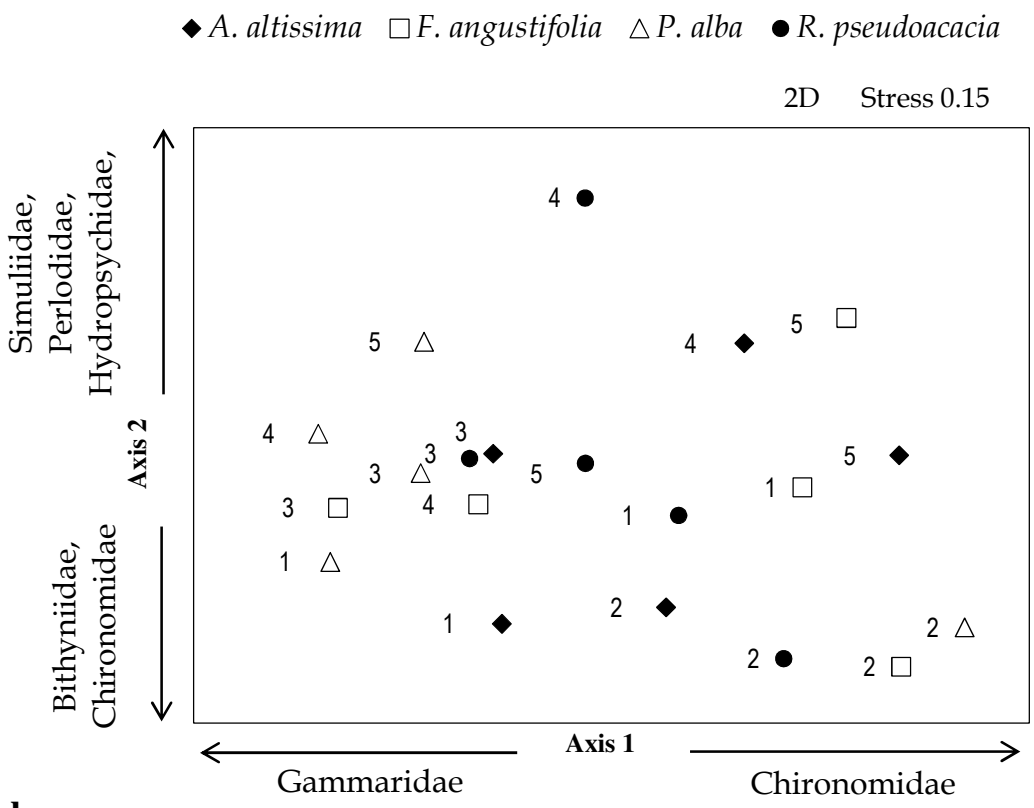


Figure 3. Mean (n = 4) density (a) and richness (b) of macroinvertebrates for each species over the study period. Black symbols indicate exotic species, white symbols native species and grey symbols empty bags (control). SE is not shown for better clarity.

The first axis of the NMDS was negatively correlated with the abundance of Gammaridae (Amphipoda) and positively with that of Chironomidae (Diptera) (Fig. 4b). Axis 2 was negatively correlated with the abundance of Bithyniidae (Mollusca) and Chironomidae and positively correlated with that of Simuliidae (Diptera), Perlodidae (Plecoptera) and Hydropsychidae (Trichoptera) (Fig. 4b) (**Anexo, foto 8**). The NMDS biplots showed that macroinvertebrates from the four leaf species appeared close to each other in the first three collections, except those of *P. alba*, which appeared separated from the other species in the first collection (Fig. 4a). On the contrary, samples of the two last collections were scattered throughout the ordination axes. In the fourth collection, after 62 days in the stream, samples corresponding to native leaves appeared clustered and separated from those corresponding to the exotic leaves. In the fifth collection, after 82 days in the stream, *P. alba* and *R. pseudoacacia* samples appeared clustered and far from *A. altissima* and *F. angustifolia* along axis 1 of the NMDS. However, perMANOVA showed that

only time significantly affected the composition of macroinvertebrate families ($F_{4, 84} = 4.09$, $p < 0.05$).

a



b

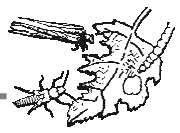
	Pearson correlation test			
	Axis 1		Axis 2	
	r	*p*	*r*	*p*
Chironomidae	0.81	**< 0.0001**	-0.64	**0.0022**
Gammaridae	-0.81	**< 0.0001**		
Simuliidae			0.73	**0.0002**
Hydropsychidae			0.8	**< 0.0001**
Bithyniidae			-0.66	**0.0016**
Perlodidae			0.62	**0.0036**

Figure 4. (**a**) Two dimensional Non Metric Multidimensional Scaling (NMDS) for C-bags using the mean relative abundances of macroinvertebrate families per leaf specie and collection time. Symbols represent different species. Black symbols indicate exotic species (*Ailanthus altissima* and *Robinia pseudoacacia*), white symbols native species (*Fraxinus angustifolia* and *Populus alba*). Numbers next to symbols designate collection times (1= 2 days, 2 = 20 days, 3 = 39 days, 4 = 62 days, 5 = 82 days). (**b**) Pearson correlation test between mean relative abundance of macroinvertebrate families and NMDS axis. Significant correlations ($p < 0.05$) are indicated in bold.

Discussion

Riparian invasion by exotic trees imply that streams receive inputs of leaves with properties that may widely differ from the native leaves, which may alter the structure and function of aquatic communities (Webster et al. 1990). Our study showed that: 1) fungal colonization was lower in exotic than in the native leaf litter and 2) the contribution of macroinvertebrates to leaf decomposition (as reflected by k_C:k_F) was more important in native than in exotic leaves, although no differences were found in macroinvertebrate density. In accordance with the *Novel Weapons Hypothesis* (NWH), fungi may colonize better the litter from those species they have co-evolved with and macroinvertebrates may have lower effect on exotic leaves because they are not pre-adapted to their secondary compounds (Callaway and Ridenour 2004; Gama et al. 2014). Besides, the greater fungal colonization may also explain the greater effect of macroinvertebrates on native than on exotic leaves, since macroinvertebrates usually prefer more preconditioned leaves by microorganisms (Wright and Covich 2005). Finally, k_C:k_F ratio, indicating the balance in the contribution of microorganisms and detritivorous macroinvertebrates, accounted for the responses to different leaf litter quality (Gessner and Chauvet 2002). This balance changed in presence of A. *altissima* and *R. pseudoacacia* leaf litter which differed in quality from that of native species.

Given that fungi and macroinvertebrates are important agents involved in leaf litter decomposition (Abelho 2001; Cornut et al. 2010), and they affected less the exotic litter, slower decomposition rates (k) would also be expected for exotic than for native leaves. However, physical-chemical differences across leaf species explained better the differences in k than their origin (exotic vs. native), in accordance with previous studies (Hladyz et al. 2009; Bottollier-Curtet et al. 2011; Casas et al. 2013). The ranking of k in the presence of macroinvertebrates was *A. altissima* = *F. angustifolia* > *P. alba* > *R. pseudoacacia*, slightly different from the ranking found in their absence (*A. altissima* > *F. angustifolia* > *P. alba* > *R.*



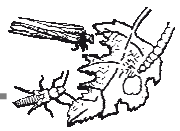
pseudoacacia). Lignin, ADF and lignin:P were good predictors of k in the presence of macroinvertebrates (k_C) and in their absence (k_F) as previously reported (Ostrofsky 1997; Hladyz et al. 2009). Besides, the leaf P and N:P ratio were also important in determining k_C, according to Hladyz et al. (2009). Physical abrasion by stream water flow is also an important factor determining leaf decomposition (Abelho 2001). For instance, the fibers, such as lignin or ADF, increase the resistance of leaf lamina to fracture, which in turn determines lower effect of water flow on k (Choong et al. 1992). Consequently, *A. altissima* and *F. angustifolia* leaves, which presented the lowest ADF and lignin concentrations, decomposed faster than leaves of the other species.

According to NWH, the presence of secondary compounds in the leaves of both exotics, such as robinetin, myricetin and quercetin in *R. pseudoacacia* (Nasir et al. 2005) and ailanthone, chaparrine, and ailanthinol B in *A. altissima* (De Feo et al. 2003), may explain their poor fungal colonization. Phytotoxic compounds of other species have been found to inhibit microbiological activity in soils (Llinares et al. 1993) and essential oils from *Eucalyptus globulus* may also delay fungal growth (Graça et al. 2002). Fungal biomass was reported as a good predictor of k (Hladyz et al. 2009) and can explain why the native litter from *P. alba* decomposed faster than that from the exotic *R. pseudoacacia,* even when they had similar fiber concentration. The high fungal biomass found in *F. angustifolia* leaves at 20 days of stream immersion was probably due to its low phenolic concentration, since phenolic compounds may depress fungal growth (Mathuriau and Chauvet 2002). Besides, fungi could take nutrients from the water column to compensate the effect of low-quality litter in streams with high nutrient levels (Gulis and Suberkropp 2003). Therefore, given that our stream was P-limited (N:P > 40), the high P content of *F. angustifolia* leaves may additionally contribute to a high colonization by fungi. Accordingly, we found that P concentration in leaves have a positive effect in decomposition rates. *F. angustifolia* leaves decomposed slower than *A. altissima* in absence (F-bags) but not in presence (C-bags) of macroinvertebrates, probably because fungal colonization in *F. angustifolia* leaves was greater in F than in C-bags. The overall

lower fungal biomass found in F-bags as compared with C-bags can be explained by two non-exclusive arguments: First, given that aquatic hyphomycetes (the main microbial decomposers in streams; Gulis and Suberkropp 2003) are sensitive to oxygen availability (Medeiros et al. 2009), a poor oxygen diffusion into F-bags, due to the smaller mesh size, may have hampered the colonization of litter by this group of microbes. Second, macroinvertebrates may enhance fungal development on leaf litter as their feces represent a source of food for fungi (Villanueva et al. 2012).

Although we found similar or greater k of *A. altissima* than that of *F. angustifolia* in the stream, Alonso et al. (2010) found the contrary in a man-made lake. This suggests higher vulnerability of *A. altissima* leaves to physical abrasion by the stream water flow. In fact, *A. altissima* presents soft leaves (Swan et al. 2008), which acquires a gelatinous consistency during decomposition (personal observation) and k of soft leaves is enhanced by the water flow (Abelho 2001). In addition, the absence of shredders in the man-made lake makes microbial decomposition more relevant (Alonso et al. 2010), which may favor decomposition of *F. angustifolia* over *A. altissima*, since, as reported above, the native may buildup greater fungal biomass than the exotic species.

Decomposition rates may vary across streams with different characteristics, such as temperature, nutrient levels, macroinvertebrates or riparian vegetation (Grubbs and Cummins 1994; Menéndez et al. 2011; Pérez et al. 2011). For instance, Casas et al. (2013) found that k was greater in streams receiving lower quality litter than in streams receiving higher quality litter from the riparian vegetation. However, the differences in k among leaves from *Alnus glutinosa* L., *Quercus pyrenaica* Wild., *Pinus radiata* D. Don., and *Platanus hispanica* Mill. ex Münchh. remained unaltered across streams, being the quality of litter the most important factor determining these differences (Casas et al. 2013). This suggests that in our study, the differences found in k among species would be extensible to other streams. Decomposition rates may also vary



among seasons due to changes in water temperature or in the structure of macroinvertebrate community (Pérez et al. 2011). The four leaf species used in our study have their leaf litterfall peaks in autumn, which makes more relevant the choice of that season under an ecological perspective.

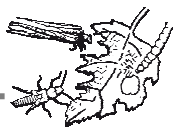
Increases in N litter concentration over the decomposition process can be attributed to microorganism's N uptake from the water column and subsequent immobilization in form of microbial biomass (Kuehn et al. 2000). Also, a faster loss of N-rich than N-poor leaf litter compounds may occur during decomposition. The great N concentration observed in *R. pseudoacacia* litter throughout the experiment suggests that the organic N concentration may increase in the stream upon invasion by this species, as reported for the N-fixing species *Elaeagnus angustifolia* (Mineau et al. 2011). Furthermore, nitrate accumulation has been reported in wetlands adjacent to *R. pseudoacacia* stands (Williard et al. 2005). Therefore, if *R. pseudoacacia* invaded riparian zones lacking native N-fixing trees, stream N would increase.

We found no differences in the relative abundance of shredders, total density and richness of macroinvertebrates across leaves of different species, suggesting that macroinvertebrates were able to use both exotic and native leaves, either as refuge or food, as reported by others (Albariño and Balseiro 2002). The density of macroinvertebrates drastically dropped after the hydropeaking event that occurred in the middle of the study period. Similarly, in a regulated stream, Mendoza-Lera et al. (2010) found that after water discharges the density and biomass of macroinvertebrates decreased, which slowed down decomposition. This suggested that hydropeaking events may affect in greater extent leaf species which decomposition is mainly determined by macroinvertebrates. This fact could be important in regulated streams, which are frequently impacted by hydropeakings.

The macroinvertebrate community highly varied during the study period across collections, but not across species. However, we can point out some different trends at final stages of decomposition, likely due to different

leaf chemistry, fungal leaf preconditioning and/or availability of remaining material (Casas and Gessner 1999). For instance, the weight of Chironomidae (collector-gatherer) family, which feed on depositional fine particulate organic matter (FPOM), was higher in litter bags with little or no remaining mass (*A. altissima* at 62 and 82 days and *F. angustifolia* at 82 days). At 62 days in the stream, Gammaridae (shredder) were dominant in native leaves contrasting with the high weight of Simuliidae, Perlodidae and Hydropsychidae in *R. pseudoacacia* leaves. The most abundant families found in *R. pseudoacacia* litter bags at 62 days corresponded to functional feeding groups which do not feed directly on leaves (i.e. collector-filterers and predators), suggesting that they used *R. pseudoacacia* leaves as a refuge or as a source of preys. By contrast, Gammaridae family consumes preferentially fungal pre-conditioned leaves (Graça et al. 1993; Foucreau et al. 2013), explaining the high weight of Gammaridae in *P. alba* leaves, which were more colonized by fungi than *R. pseudoacacia*. This result may also account for the co-evolution of Gammaridae with the associated fungal species in *P. alba* leaves (according to NWH), since fungal species composition in leaves determined the feeding activity of a variety of shredders (Arsuffi and Suberkropp 1989). Therefore, Gammaridae family may be negatively affected if *P. alba* was eventually displaced by exotic trees.

Since differences in leaf decomposition among species were species-specific, the consequences of the exotic species on k will depend on the prevailing native species of the invaded ecosystem. For instance, greater impact on k can be expected if *A. altissima* replace vegetation dominated by *P. alba* (decomposition would be accelerated) and if *R. pseudoacacia* replace vegetation dominated by *F. angustifolia* (decomposition would be slowed down). Given the fast decomposition of *A. altissima,* a riparian invasion by this species may reduce the time of food availability to shredder macroinvertebrates (Wallace et al. 1999) affecting their life cycle, particularly in those species where the major



growth period occurs in late autumn and winter (e.g. many Trichoptera) (Anderson and Cummins 1979).

Conclusions

In conclusion, this study shows the potential impact of the displacement of native vegetation (characterized by *P. alba* and/or *F. angustifolia*) by the exotics *A. altissima* and/or *R. pseudoacacia* in a headwater stream ecosystem. The exotic *A. altissima* may increase decomposition rates while *R. pseudoacacia* would have the reverse effect. The intensity of these impacts will depend on the native vegetation that they replace. Leaf species and their chemical characteristics (mainly fiber concentration and phosphorous) controlled within-stream leaf decomposition. Moreover, leaves of both exotic species were less colonized by fungi which in turn may affect macroinvertebrates that feed on preconditioned leaves. Gammaridae family could be one of the most affected because tended to have higher weight on macroinvertebrate community colonizing leaves of *P. alba* than that of other species. The Novel Weapons Hypothesis could explain the lower fungal colonization and lower effect of macroinvertebrates on litter decomposition in exotic than in native species, while contrasting decomposition rates among species could mainly be explained by the litter quality.

Acknowledgements

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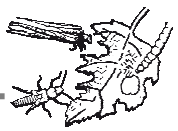
Supplementary Information

Table S1. Mean relative abundance of macroinvertebrate families occurring in at least 5 % of the coarse litter bags, per species and collection time (2, 20, 39, 62 and 82 days). FFG: Functional Feeding Groups; PR: predator; SC: scraper; SH: shredder; CG: Collector-Gatherer; CF: Collector-Filterer.

			Ailanthus altissima					*Fraxinus angustifolia*					*Populus alba*					*Robinia pseudoacacia*				
Order	**Family**	**FFG**	2 d	20 d	39 d	62 d	82 d	2 d	20 d	39 d	62 d	82 d	2 d	20 d	39 d	62 d	82 d	2 d	20 d	39 d	62 d	82 d
Turbelaria	Planariidae	PR	0.022	0.05	0.015	0.012	0.023	0	0.028	0.025	0.023	0.009	0	0.171	0.045	0.029	0.075	0	0.119	0.013	0.025	0.056
Mollusca	Bithyniidae	SC	0.186	0.239	0.052	0.025	0.012	0.063	0.207	0.153	0.115	0	0	0.124	0.083	0	0.035	0.063	0.273	0.08	0.091	0.04
Aracnida	Hydracarina	PR	0.037	0.013	0.026	0.004	0.016	0	0.011	0	0.032	0.005	0.023	0.025	0.049	0.028	0.003	0.063	0.013	0.053	0	0.03
Amphipoda	Gammaridae	SH	0.217	0.278	0.384	0.135	0.097	0.271	0.175	0.422	0.398	0.067	0.485	0.117	0.411	0.435	0.292	0.275	0.163	0.353	0.05	0.3
Ephemeroptera	Baetidae	CG	0.095	0.026	0.101	0.32	0.139	0.333	0.008	0	0.15	0.333	0.21	0.004	0.069	0.009	0.027	0.225	0	0.03	0	0.053
Ephemeroptera	Heptageniidae	CG	0	0	0.006	0	0	0	0	0.075	0	0	0	0	0	0.029	0.02	0	0.003	0.004	0	0.002
Plecoptera	Nemouridae	SH	0	0.004	0.013	0.003	0.028	0	0.008	0	0.012	0.02	0	0.001	0.016	0	0	0	0.004	0	0	0.044
Plecoptera	Capniidae	SH	0	0	0	0	0	0	0.002	0.05	0	0	0	0	0	0	0.068	0	0	0.011	0.091	0.009
Plecoptera	Perlodidae	PR	0	0	0.006	0.007	0.003	0	0	0	0.009	0.013	0	0.002	0.031	0.063	0	0	0	0.021	0.136	0.01
Coleoptera	Scirtidae	SH	0	0	0.006	0.004	0	0	0	0	0.018	0.003	0	0	0	0	0.018	0	0	0	0	0.041
Trichoptera	Hydropsychidae	CF	0.012	0.018	0.09	0.161	0.201	0	0.004	0.013	0.066	0.172	0.021	0.03	0.038	0.107	0.309	0	0.036	0.052	0.261	0.157
Trichoptera	Psychomyiidae	SH	0.012	0	0	0	0.014	0	0	0	0	0	0	0	0	0	0.034	0	0.003	0	0	0.014
Trichoptera	Rhyacophilidae	PR	0	0	0	0	0.008	0	0	0	0	0.005	0	0.005	0.005	0	0	0	0	0.008	0	0
Diptera	Chironomidae	CG	0.147	0.354	0.157	0.164	0.389	0.333	0.528	0.128	0.135	0.196	0.177	0.485	0.126	0.119	0.094	0.25	0.383	0.172	0.075	0.203
Diptera	Simuliidae	CF	0.011	0.01	0.144	0.163	0.058	0	0.018	0.134	0.037	0.164	0	0.004	0.128	0.18	0.023	0	0.003	0.201	0.27	0.033
Diptera	Ceratopogonidae	PR	0.262	0.009	0	0.003	0.013	0	0.012	0	0.004	0.011	0.083	0.032	0	0	0.002	0.125	0	0	0	0.009



Capítulo 6

Resumen de los resultados de la Tesis

Sólo una cosa vuelve un sueño imposible: el miedo a fracasar.

Paulo Coelho

Fotografía: Tramo del Río Henares cerca de la población de Chiloeches (Guadalajara, España).
Por: Silvia Medina Villar

Photography: Stretch of the Henares River near Chiloeches village (Guadalajara, Spain).
By: Silvia Medina Villar

Resumen de los resultados de la Tesis

Los estudios realizados en esta tesis doctoral permiten un mejor conocimiento de los impactos de las especies invasoras, *Ailanthus altissima* y *Robinia pseudoacacia*, en los ecosistemas de ribera y fluviales. Los impactos en los ciclos de nutrientes fueron más claros que en las comunidades, ya sea de plantas, microorganismos o macroinvertebrados.

A nivel de ecosistema

En relación al proceso de caída de la hojarasca, no se observaron diferencias significativas en la cantidad de la hojarasca producida en bosques invadidos por las especies exóticas *A. altissima* y *R. pseudoacacia* y la producida en bosques no invadidos (Tabla 1). La dinámica temporal de caída de hojarasca se caracterizó por la presencia de un pico de caída principal en los meses de otoño, ya que todas las especies arbóreas de estudio, tanto las exóticas invasoras como las nativas (*Fraxinus angustifolia* y *Populus alba*) son especies de hoja caduca. No obstante, la dinámica temporal de caída de diferentes órganos senescentes varió entre bosques invadidos y no invadidos (dominados por los árboles nativos *F. angustifolia* y *P. alba*) (Tabla 1, *Capítulo 2*). Específicamente, en los boques invadidos las flores cayeron dos meses más tarde que en bosques no invadidos. Además, en primavera tardía y verano aparecieron picos de caída de hojas y/o órganos reproductores de la planta en bosques invadidos pero no en nativos. Los picos adicionales de hojarasca implicaron aportes adicionales de nitrógeno (N) y fósforo (P) en el suelo. La calidad de la hojarasca varió significativamente entre las especies de árboles. En general, las diferentes fracciones de hojarasca producidas en los bosques invadidos por *A. altissima* y *R. pseudoacacia* presentaron las concentraciones más altas de N y P mientras que las fracciones producidas en los bosques dominados por la especies nativa *P. alba* mostraron las concentraciones más bajas (Tabla 1, *Capítulo 2*). Esto podría haber determinado, en parte, las mayores concentraciones de nutrientes (N y P total) del suelo observadas bajo el dosel de los árboles invasores que bajo el dosel de *P. alba* (Tabla 1, *Capítulo 2*). Los suelos bajo el dosel

de las especies invasoras *A. altissima* y *R. pseudoacacia* también mostraron mayor concentración de N que los suelos bajo el dosel de la especie nativa *F. angustifolia* (Tabla 1, *Capítulo 2*).

Otras características que difirieron entre las hojas senescentes de las distintas especies de árboles fueron la concentración de lignina, de fibra ácido detergente (FAD) y de compuestos fenólicos totales, así como los ratios N:P, lignina:P y lignina:N. Las hojas senescentes de *A. altissima* y *F. angustifolia* fueron más parecidas entre sí en cuanto a su menor contenido en fibras (lignina y FAD) que las hojas senescentes de *P. alba* y *R. pseudoacacia* (Tabla 1). Esto pudo facilitar la descomposición de la hojarasca de *A. altissima* y *F. angustifolia* en el ecosistema fluvial. Las hojas senescentes de *A. altissima* se descompusieron más rápidamente y las de *R. pseudoacacia* más lentamente que las de las especies nativas (Tabla 1, *Capítulo 5*). Los resultados, tanto del muestreo de suelo en campo como del experimento de invernadero, mostraron la especie invasora *R. pseudoacacia* puede aumentar las concentraciones de N mineral del suelo, mientras que la invasora *A. altissima* puede disminuir el N orgánico (Tabla 1, *Capítulo 3*). En general se observaron mayores tasas netas de mineralización del N y mayor actividad PME en suelos de sitios invadidos por *R. pseudoacacia* que en suelos de sitios no invadidos (dominados por *P. alba*) (Tabla 1, *Capítulo 3*). Sin embargo, el efecto contrario se observó en sitios invadidos por *A. altissima* (Tabla 1, *Capítulo 3*)

Resultados a nivel de comunidad y especie

Apoyando la *Hipótesis de las Nuevas Armas* se encontraron los siguientes resultados a nivel de comunidad y especie. Por un lado, la colonización fúngica de hojas procedentes de árboles exóticos fue menor que la de hojas procedentes de árboles nativos (Tabla 1, *Capítulo 5*). Por otro lado, se observó un mayor efecto de los macroinvertebrados en el procesamiento de hojas exóticas respecto de las nativas (Tabla 1, *Capítulo 5*). Además, la comunidad de bacterias del suelo difirió entre sitios invadidos por *A. altissima* y sitios no invadidos (dominados por la

especie nativa *P. alba*), siendo la diferencia más leve para la comparación de *R. pseudoacacia* con *P. alba* (Tabla 1, *Capítulo 3*).

Los efectos alelopáticos ocasionados por extractos de hojas senescentes (de aquí en adelante "extractos") sobre la germinación y crecimiento radicular de especies de plantas nativas del sotobosque dependieron enormemente del sustrato utilizado, siendo los efectos mayores en papel de germinación que en suelo. En general, cuando el papel de germinación fue utilizado como sustrato, los extractos de *A. altissima* causaron una disminución menor del porcentaje final de germinación (G) de plantas del sotobosque que los extractos de especies nativas (Tabla 1, *Capítulo 4*). También usando papel de germinación como sustrato, la reducción del índice de la velocidad de germinación (IVG) de las especies del sotobosque por los extractos de *A. altissima* fue menor que la producida por los extractos de *P. alba* (Tabla 1, *Capítulo 4*). Sin embargo, los extractos de *A. altissima* disminuyeron la longitud de radícula (LR) más que los extractos de *F. anfustifolia* sobre papel de germinación (Tabla 1, *Capítulo 4*). Los extractos de *R. pseudoacacia* redujeron más intensamente la LR de las especies del sotobosque que los extractos de las especies nativas cuando el papel de germinación fue utilizado como sustrato (Tabla 1, *Capítulo 4*). Usando suelo como sustrato, los extractos de *R. pseudoacacia* fueron más efectivos reduciendo el G y el IVG de las especies del sotobosque que los extractos de la especie nativa *P. alba* (Tabla 1, *Capítulo 4*).

Tabla 1. Resumen de los resultados de la tesis para cada una de las variables estudiadas en las comparaciones de la especies invasoras *Ailanthus altissima* (**Aa**) y *Robinia pseudoacacia* (**Rp**) con las nativas *Populus alba* (**Pa**) y/o *Fraxinus angustifolia* (**Fa**). El valor de cada variable en la especie invasora resultó significativamente mayor (>), menor (<), distinta cuando no aplicaba el sentido de la diferencia (≠) o igual (=) que en la especie nativa. N: nitrógeno; P: fósforo; OM: materia orgánica; FAD: fibra ácido detergente; PME: Fosfomonoesterasa; G: germinación; IVG: índice de velocidad de germinación; LR: Longitud de radícula.

					Aa		Rp	
Nivel de organización		**Componente o proceso del ecosistema**	**Variables medidas**		**Fa**	**Pa**	**Fa**	**Pa**
		Dinámica temporal de caída de diferentes componentes de la hojarasca (*Capítulo 2*)			≠	≠	≠	≠
		Cantidad de hojarasca (por superficie y unidad de área basimétrica de los árboles de plots de 300 m² (*Capítulo 2*)	Hojas (g m^{-4})		=	=	=	=
			Flores (g m^{-4})		=	=	=	=
			Frutos (g m^{-4})		=	=	=	=
			Ramas (g m^{-4})		=	=	=	=
			Total (g m^{-4})		=	=	=	=
			Total en forma de N (g m^{-4})		=	=	=	=
			Total en forma de P (g m^{-4})		=	=	=	=
		Calidad de la hojarasca (*Capítulo 2*)	Hojas completas	N (%)	=	>	=	>
				P (%)	=	=	=	=
			Flores	N (%)	=	>	=	>
				P (%)	=	>	=	=
			Frutos	N (%)	=	>	>	>
				P (%)	=	=	>	>
			Ramas	N (%)	=	=	=	=
				P (%)	=	=	<	=
		Calidad de la hojarasca (Se utilizó la lámina de la hoja: Foliolos de las hojas compuestas y hojas simples sin peciolos) (*Capítulo 5*)	N (%)		>	>	>	>
			P (%)		=	=	<	=
			Lignina (%)		=	<	>	=
			FAD (%)		=	<	>	<
			TPC (%)		>	=	>	=
			N:P		>	=	>	>
			Lignin:N		<	<	=	<
			Lignin:P		=	<	>	=
		Tasa de descomposición de la hojarasca en el río (*Capítulo 5*)	k_C		=	>	<	<
			k_F		>	>	<	<
			k_C:k_F		<	<	<	<
		Características del suelo (Las unidades de N, P y OM están en función de la densidad de árboles en plots de 300 m²) (*Capítulo 2*)	N (% m^{-2})		>	>	>	>
			P (% m^{-2})		=	>	=	>
			OM (% m^{-2})		=	>	>	>
			pH		=	=	=	=
		Características del suelo[1] (*Capítulo 3*)	N (%)			<(<)[1]		>(=)[1]
			P (%)			=(=)[1]		=(=)[1]
			Amonio (mg Kg^{-1})			<(=)[1]		=(>)[1]
			Nitrato (mg Kg^{-1})			>(=)[1]		>(>)[1]
			N mineral (mg Kg^{-1})			=(=)[1]		>(>)[1]
			MO (%)			<(=)[1]		=(=)[1]
			pH			=(=)[1]		=(=)[1]
		Mineralización del N y P del suelo (*Capítulo 3*)	Tasa total (µg g suelo^{-1} día^{-1})			<(=)[1]		=(<)[1]
			Tasa de amonificación (µg g suelo^{-1} día^{-1})			=(=)[1]		>(<)[1]
			Tasa de nitrificación (µg g suelo^{-1} día^{-1})			<(=)[1]		=(<)[1]
			Actividad PME (µmol *p*-NP g suelo^{-1} h^{-1})			<(=)[1]		>(=)[1]
			Nitrato:Amonio			>(=)[1]		>(=)[1]
	Comunidad y especie	Comunidades de las hojas en descomposición (*Capítulo 5*)	Biomasa fúngica (mg g^{-1})		<	<	<	<
			Macroinvertebrados acuáticos		=	≠	=	≠
		Comunidad de bacterias del suelo (*Capítulo 3*)				≠(=)[1]		=(=)[1]
		Especies de plantas del sotobosque (*Capítulo 4*)	Magnitud del efecto reductor sobre las siguientes variables:	G (%)	<(=)[2]	<(=)[2]	=(=)[2]	=(>)[2]
				IVG	=(=)[2]	<(=)[2]	=(=)[2]	=(>)[2]
				LR (mm)	>(=)[2]	=(=)[2]	>(=)[2]	>(=)[2]

[1]Fuera del paréntesis: resultado de un muestreo de campo en el que se controla la cantidad de hojarasca en el suelo y el tamaño del árbol. Entre paréntesis: resultado de un experimento de invernadero con plantas de 6 meses.
[2]Sustrato: papel de germinación (fuera del paréntesis) y suelo (dentro del paréntesis)

Capítulo 7

Discusión general

Me lo contaron y lo olvidé; lo vi y lo entendí; lo hice y lo aprendí.

Confucio

Fotografía: A la izquierda de la fotografía, la especie nativa, *Populus alba*, y a la derecha la especie exótica invasora, *Ailanthus altissima.*
Por: Luciano Pataro

Photography: On the left of the photography, the native species, *Populus alba* and on the right the exotic invasive species, *Ailanthus altissima*.
By: Luciano Pataro

Discusión general

Esta Tesis Doctoral aporta una visión global de los impactos ecológicos de dos especies de árboles exóticos invasores, *Ailanthus altissima* y *Robinia pseudoacacia,* en los ecosistemas fluviales y de ribera del Centro de la Península Ibérica. Los resultados indican que ambas especies pueden producir impactos tanto a nivel de ecosistema, como de comunidad y especie. El conocimiento de estos impactos supone una información esencial para la elaboración de los análisis de riesgo de estas especies en zonas donde son potencialmente invasoras (Capdevila et al. 2006). Además, ya que los recursos para llevar a cabo la gestión de las especies exóticas invasoras (EEI) por parte de las Comunidades Autónomas son muy limitados (Capdevila et al. 2006), un mejor conocimiento de la severidad de las consecuencias ecológicas producidas por *A. altissima* y *R. pseudoacacia,* en comparación con las producidas por otras EEI, ayudará a tomar decisiones en cuanto a la priorización o no de su gestión.

A través de la hojarasca las especies de plantas afectan a diferentes componentes del ecosistema, como los nutrientes del suelo, las especies de plantas del sotobosque, los organismos descomponedores o las comunidades microbianas (Vitousek 1984; Facelli y Pickett 1991; Wallace et al. 1997; Xiong y Nilsson 1999). La entrada de especies exóticas de plantas en los ecosistemas puede suponer cambios en la cantidad, calidad y dinámica de caída de la hojarasca, afectando, por tanto a otros componentes del ecosistema. En esta memoria de Tesis Doctoral, se pone de manifiesto como la hojarasca producida por *A. altissima* y *R. pseudoacacia* causa impactos en los ecosistemas fluviales y de ribera a diferentes niveles (ecosistema, comunidad y especie). En este sentido, nuestros resultados mostraron que respecto de los bosques nativos (dominados por las especies *P. alba* y *F. angustifolia*), los bosques invadidos por *A. altissima* y *R. pseudoacacia* produjeron cantidades similares de hojarasca pero de diferente calidad (contenido en nutrientes) y dinámica temporal de caída (*Capítulo 2*). Estas diferencias probablemente determinaron cambios en otros

componentes del ecosistema. Por un lado, la mayor concentración de nitrógeno (N) y fósforo (P) de la hojarasca de las EEI pudo determinar, en parte, la mayor concentración de dichos nutrientes en los suelos de los bosques invadidos (*Capítulo 2*). No obstante, otros mecanismos pueden explicar también los impactos de las EEI en determinadas propiedades del suelo, como por ejemplo, la fijación de N atmosférico, que probablemente permitió a *R. pseudoacacia* aumentar el N mineral del suelo (*Capítulo 3*). Por otro lado, determinados compuestos químicos específicos de la hojarasca exótica pudieron afectar a las comunidades de hongos del ecosistema fluvial, a las comunidades de bacterias del suelo y a las especies de plantas del sotobosque (*Capítulos 3, 4 y 5*), según la *Hipótesis de las Nuevas Armas* (HNA) (Callaway y Ridenour 2004). Por último, las diferentes características químicas de la hojarasca exótica respecto de la nativa determinaron diferentes tasas de descomposición en el ecosistema fluvial (*Capítulo 5*).

El estudio de los diferentes impactos de *A. altissima* y *R. pseudoacacia* sobre los ecosistemas de ribera y fluviales se realiza en los diferentes estudios de esta Tesis Doctoral mediante la comparación de los valores de diferentes variables entre las EEI y las especies nativas *P. alba* y/o *F. angustifolia.* Esto es importante para relativizar los impactos de las especies invasoras (Shannon-Firestone y Firestone 2015). Algunos de los impactos ecológicos observados en esta memoria para *A. altissima* y *R. pseudoacacia* tuvieron el mismo sentido, mientras que otros impactos variaron enormemente en magnitud y sentido en función de la especie invasora, la especie nativa de comparación y el sitio de estudio. Por ejemplo, en el ecosistema fluvial ambas EEI causaron una reducción de la colonización fúngica de la hojarasca, pero *A. altissima* aumentó y *R. pseudoacacia* disminuyó las tasas de descomposición de la hojarasca respecto de las especies nativas (*Capítulo 5*). Esto pone de manifiesto la enorme dependencia del contexto en el estudio de las invasiones biológicas, lo que hace muy difícil hacer generalizaciones sobre los impactos que producen las EEI en los ecosistemas (Ricciardi et al. 2013).

Las características iniciales de cada sitio antes de la invasión también influyen en la producción de impactos, especialmente aquellos que afectan a los ciclos de nutrientes (Dassonville et al. 2008). Así, las EEI tienden a aumentar las concentraciones de nutrientes del suelo cuanto menor es su concentración inicial y viceversa (Dassonville et al. 2008). En nuestro estudio, la influencia del sitio se observó en el impacto de *A. altissima* en el N total del suelo, el cual dependió de los niveles iniciales de N en la zona no invadida (considerada como la situación pre-invasión). Justamente, cuando las concentraciones de N del suelo pre-invasión fueron más bajas, *A. altissima* aumentó el N total del suelo (*Capitulo 2*), mientras que lo disminuyó cuando el valor inicial pre-invasión fue mayor (*Capítulo 3*). Esto podría indicar la capacidad de *A. altissima* de adecuar las características del suelo a valores óptimos para su crecimiento. De acuerdo con esto, *A. altissima* aumentó el N del suelo en sitios cuya concentración de N del suelo era más baja que en los suelos analizados en esta Tesis Doctoral (Vilà et al. 2006; Gómez-Aparicio y Canham 2008b). Nuestros resultados resaltan la relevancia de estudiar cada especie invasora en su contexto particular de invasión, ya que los impactos pueden variar de un sitio a otro.

Se discutirán a continuación de forma más detallada los impactos de *A. altissima* y *R. pseudoacacia* a nivel de ecosistema, comunidad y especie.

Impactos a nivel de ecosistema

Las consecuencias ecológicas de *A. altissima* y *R. pseudoacacia* a nivel de ecosistema se manifestaron en cambios en el momento de entrada de la hojarasca en el suelo, en la calidad de la hojarasca, en las tasas de descomposición de la hojarasca en medio acuático, y en los contenidos y tasas de mineralización de los nutrientes del suelo.

Las diferencias observadas en la dinámica de caída de hojarasca entre bosques nativos e invadidos se revelaron en picos adicionales de caída de hojas y flores en verano y primavera tardía producidos por las EEI (*Capítulo 2*). Estos

picos suponen una entrada importante de nutrientes al suelo en períodos en los que el aporte de hojarasca procedente de los bosques nativos es escaso. La caída temprana de hojas puede deberse a que las EEI no están adaptadas al estrés hídrico estival típico de los climas mediterráneos. Estas hojas que caen de forma prematura normalmente contienen una mayor concentración de nutrientes debido a un menor tiempo para su re-movilización (Killingbeck 1996). Además, la caída de flores de los árboles invasores se produjo en Junio, dos meses después que la de las especies nativas. En el caso de *R. pseudoacacia*, se ha demostrado que las flores suponen un importante aporte de P en el suelo (Lee et al. 2011), nutriente altamente demandado por especies fijadoras de N, como R. *pseudoacacia* (Reinsvold y Pope 1987; Makoi y Ndakidemi 2008). Por otro lado, la hojarasca que cae al suelo en verano podría descomponerse más lentamente, ya que la actividad microbiana y de invertebrados detritívoros es más lenta debido a la escasez de agua (Cortez 1998). Esto puede dar lugar a un aumento del tiempo durante el cual los nutrientes orgánicos (N y P) permanecen en el suelo, contribuyendo a su aumento en los bosques invadidos respecto de los nativos (Kirschbaum 2010).

Los cambios en el momento de caída de la hojarasca en los ecosistemas de ribera invadidos por *A. altissima* y *R. pseudoacacia* pueden afectar también a las comunidades de detritívoros tanto acuáticos como terrestres y de especies de plantas del sotobosque (Facelli y Picket 1991; Abelho y Graça 1996). Por ejemplo, los picos adicionales de caída de hojarasca suponen una alteración de los periodos de disponibilidad de alimento para las comunidades de detritívoros de los ecosistemas fluviales y de ribera (Takeda 1987; Ponge et al. 1993; Wallace et al. 1997). Por otro lado, la hojarasca acumulada en sitios invadidos en primavera tardía y verano puede suponer una barrera física para la germinación de semillas de determinadas especies de plantas (Facelli y Picket 1991). Esto es especialmente relevante para especies como la nativa *P. alba,* siendo una especies que no presenta banco de semillas, germina en Mayo-Junio y cuyas semillas son de pequeño tamaño (Prada y Arzipe 2008). Esto podría dificultar el establecimiento y dispersión de *P. alba* en sitios invadidos por *R.*

pseudoacacia y *A. altissima*. Además, en comparación con la dispersión puntual de los frutos observada en los bosques nativos dominados por la especie *P. alba*, la dispersión de frutos prolongada a lo largo del año en los bosques invadidos (*Capítulo 2*) implica una mayor probabilidad de establecimiento de las EEI. Otras especies de plantas herbáceas (anuales, bienales y perennes) pueden verse también afectadas por la hojarasca caída en primavera, como ha sido reportado en comunidades dominadas por la especie *Solidago canadensis* L. (Carson y Peterson 1990). Sin embargo, el impacto de la hojarasca sobre las especies de plantas del sototobosque depende del momento del ciclo vital en el que se encuentren. Por ejemplo, se ha observado que la barrera física de hojarasca perjudicó la emergencia inicial de las plántulas en otras comunidades de la especie *Cercocarpus ledifolius* Nutt. pero favoreció la supervivencia de las plántulas de un año de edad (Ibañez y Schupp 2002). La hojarasca puede también cambiar las condiciones micro-climáticas del suelo, limitando la pérdida de humedad y suavizando las temperaturas (Facelli y Picket 1991). Esto puede favorecer a determinadas especies de plantas, como *Carduus nutans* L. (Hamrick y Lee 1987), y detritívoros como algunos milpiés, anfípodos, lombrices, pseudoescorpiones e isópodos que prefieren ambientes oscuros y húmedos (Lindsay y French 2006). Por tanto, las diferencias en la dinámica de caída temporal de la hojarasca observadas en esta Tesis Doctoral pueden condicionar cambios en la estructura de las comunidades de plantas y detritívoros.

La concentración de N y P de la hojarasca de árboles exóticos y nativos fue consistente con la encontrada en los suelos bajo el dosel de cada una de las especies. Así el contenido en N y P de la hojarasca y del suelo fue el más elevado en *R. pseudoacacia* y el más bajo en *P. alba* (*Capítulo 2*). La menor eficiencia en el uso del N de *A. altissima* y *R. pseudoacacia* respecto de las especies nativas documentada en un estudio previo (González-Muñoz et al. 2013) explica que sus hojarascas presenten mayor concentración de N. Sin embargo, la concentración de N de las hojas senescentes varió dentro de la misma especie en nuestro estudio. Por ejemplo, en el *Capítulo 5*, la

concentración de N de las hojas senescentes de *A. altissima* y *R. pseudoacacia* fue mayor que la de las especies nativas, *P. alba* y *F. angustifolia,* mientras que en el *Capítulo 2* la diferencia solo fue significativa para la comparación de las EEI con *P. alba.* Esta inconsistencia indica diferencias intra-específicas en la concentración de N de la hojarasca de las especies estudiadas. Estas diferencias intra-específicas se deben a que las especies responden a los cambios en las condiciones ambientales, adaptándose a ellos o aclimatándose (Albert et al. 2011). Por ejemplo, se han observado diferencias en la composición química de de las hojas de las especies *Castanea sativa* Mill., *Quercus robur* L. y *Fagus sylvatica* L. en función de la fertilidad del suelo (Sariyildiz y Anderson 2005). Además, la concentración de carbono (C), N y P de las hojas senescentes de la especie *Populus deltoides* ssp. *wislizenii* varió en función de la hidrología de la llanura de inundación y de la fertilidad del agua (Tibbets y Molles 2005). Por tanto, la variabilidad intra-específica en la concentración de nutrientes de las hojas de las especies estudiadas en esta Tesis Doctoral puede deberse a que las hojas se recolectaron en poblaciones de sitios con diferente fertilidad o humedad del suelo. Las diferencias en la calidad de las hojas entre poblaciones de la misma especie pueden implicar cambios intra-específicos en determinados procesos del ecosistema. Por ejemplo, las hojas senescentes de la especie arbórea *Alnus glutinosa* Gaertn. presentaron mayores concentraciones de P y menores de lignina cuando pertenecían a poblaciones de latitudes más altas, lo determinó mayores tasas de descomposición de las hojas senescentes de latitudes más altas (Lecerf y Chauvet 2008). Por tanto, la variabilidad intra-específica en las características de las hojas senescentes debe ser tenida en cuenta en futuros estudios, ya que puede modificar los efectos que las especies tienen en determinados procesos del ecosistema, como la descomposición de la hojarasca.

En esta Tesis Doctoral se pone de manifiesto que la invasión de los ecosistemas de ribera por *A. altissima* produce una aceleración de las tasas de descomposición de la hojarasca en el ecosistema fluvial, mientras que la invasión por *R. pseudoacacia* produce el efecto contrario. Las características de las hojas senescentes que mejor determinaron la velocidad de descomposición

en el ecosistema fluvial fueron la concentración de lignina (%), fibra ácido detergente (FAD) (%), P (%) y los ratios N:P y lignina:P, las cuales difirieron entre las especies de estudio independientemente de su origen exótico o nativo (*Capítulo 5*). Estos resultados concuerdan con los resultados encontrados en el estudio de Hladyz y colaboradores (2009) para seis especies nativas y nueve exóticas de árboles en un ecosistema fluvial. La concentración de fibras, como la lignina y la FAD, aumenta la resistencia de la hojarasca a la rotura (Choong et al. 1992). Esto explica que la hojarasca de *A. altissima* y *F. angustifolia*, con menor concentración de FAD y lignina, se descompusiera más rápido que la de las otras especies. La elevada fragilidad de las hojas senescentes de *A. altissima* (Swan et al. 2008) hace que éstas sean más sensibles al efecto de la corriente unidireccional del río, contribuyendo también a su rápida descomposición. En este sentido, en un ecosistema fluvial lentico (sin corriente unidireccional) la descomposición de *A. altissima* fue menor que la de *F. angustifolia* (Alonso et al. 2010) mientras que sucedió lo contrario en el ecosistema fluvial lotico estudiado en esta Tesis Doctoral (*Capítulo 5*). Además, la degradación de la lignina de las hojas requiere enzimas específicas presentes en determinados microorganismos como los hongos (Pozo et al. 2009). La rápida descomposición de la hojarasca de *A. altissima* podría limitar el periodo de disponibilidad de alimento para los organismos descomponedores del ecosistema fluvial, sobre todo para aquellos cuyo periodo de crecimiento ocurre principalmente en invierno, como por ejemplo varias especies de tricópteros (Anderson y Cummins 1979). Por otro lado, la elevada concentración de N de la hojarasca *R. pseudoacacia* en diferentes grados de descomposición podría ocasionar el aumento de N orgánico en el ecosistema fluvial. Además, la baja concentración de P en las hojas de *R. pseudoacacia* restringe la disponibilidad de P para los microorganismos, lo que es especialmente relevante en ecosistemas fluviales limitados por P, ya que los hongos del río tendrían limitada la asimilación del P tanto de las hojas como de la columna de agua (Gulis y Suberkropp 2003). Por último, los invertebrados del río también podrían verse afectados por la peor calidad (baja concentración de P y elevada concentración de lignina) de las hojas senescentes de *R.*

pseudoacacia, ya que dicha calidad se ha sugerido como un limitante para los detritívoros acuáticos (Tibbets y Molles 2005)

La capacidad de las especies fijadoras de N_2 para producir impactos en el ciclo de nutrientes, sobretodo en el del N, ha sido ampliamente estudiada y revisada (Liao et al. 2008; Vilà et al. 2011; Castro-Díez et al. 2014b). Esto se pone de manifiesto en esta Tesis Doctoral con la invasión de los ecosistemas de ribera por la especie fijadora de N_2, *R. pseudoacacia*. En este sentido, los suelos de los ecosistemas invadidos por esta especie presentaron mayor concentración de N total y mineral, y mayores tasas de mineralización neta del N y el P (*Capítulos 2 y 3*) que los suelos de ecosistemas no invadidos (dominados por las especies nativas *F. angustifolia* y *P. alba*), lo que concuerda con los resultados obtenidos por otros estudios en ecosistemas dominados por especies de *Pinus* y *Quercus* (Rice et al. 2004; Malcolm et al. 2008; Von Holle et al. 2013). La mayor concentración de N total y MO en los suelos bajo el dosel de *R. pseudoacacia* que en los suelos bajo el dosel de las especies nativas puede explicarse por la elevada concentración de N y la menor tasa de descomposición de la hojarasca de *R. pseudoacacia* respecto de la de las especies nativas *Ulmus minor*, *F. angustifolia* y *P. alba* (Castro-Díez et al. 2009; Alonso et al. 2010; Medina-Villar et al. 2015b). La lenta descomposición de la hojarasca de *R. pseudoacacia* permite su acumulación en el suelo. Apoyando esta explicación, los resultados del experimento de invernadero muestran que las plántulas de *R. pseudoacacia* con solo 6 meses no produjeron apenas hojarasca y por tanto no aumentaron el N total del suelo ni la MO (*Capítulo 3*). El C y el N orgánico son sustratos necesarios para que las bacterias amonificantes produzcan amonio (Marschner y Rengel 2007). De hecho, la mineralización bruta del N está positivamente relacionada con el C y el N del suelo (Booth et al. 2005), lo que explica las mayores tasas netas de amonificación en los suelos de *R. pseudoacacia*. Del mismo modo, la menor tasa de nitrificación en los suelos de ecosistemas invadidos por *A. altissima* puede responder a la menor concentración de amonio en el suelo, que es el sustrato para las bacterias nitrificantes (Marschner y Rengel 2007).

La mayor concentración de N mineral en los suelos de ecosistemas invadidos por *R. pseudoacacia* puede atribuirse a su capacidad para fijar N_2, que implica la liberación directa de amonio al suelo por los exudados de raíz (Zahran 1999; Fustec et al. 2010). El efecto de aumento del N mineral se observó en el experimento de invernadero con plantas de *R. pseudoacacia* de tan solo 6 meses de edad, las cuales presentaban ya nódulos de fijación de N_2 (observación personal del autor) (**Anexo, foto 4**). El aumento de nitrato por especies fijadoras de N, como *R. pseudoacacia,* puede favorecer la presencia de especies nitrófilas y exóticas en bosques invadidos (Von Holle et al. 2006, 2013; Grove et al. 2015) y perjudicar el crecimiento de otras especies nativas (Vitousek et al. 1997; Haubensak y Parker 2004). Además, se ha documentado que el aumento de la concentración de nitrato en los suelos de *R. pseudoacacia* puede perdurar durante al menos 14 años tras la eliminación de la especie debido probablemente al mantenimiento de elevadas tasas netas de nitrificación en el suelo (Von Holle et al. 2013). Asimismo, la elevada movilidad de la molécula de nitrato en el suelo hace que pueda llegar fácilmente a los acuíferos y los ecosistemas fluviales (Cameron et al. 2013). De hecho, en los Montes Apalaches, área nativa de *R. pseudoacacia*, se encontraron mayores concentraciones de nitrato en los ríos en cuya cuenca se encuentra *R. pseudoacacia* (Williard et al. 2005). Se ha observado también mayor concentración de nitrato en aguas subterráneas de sitios dominados por la especie fijadora de N_2 *Acacia saligna* (Labill.) Wendl. respecto de los sitios dominados por vegetación sin la capacidad de fijar N_2 (Jovanovic et al. 2009).

La actividad de la enzima fosfomonoesterasa (PME) en el suelo, la cual regula la transformación de P orgánico a inorgánico (Nannipieri et al. 2011), fue mayor en suelos de ecosistemas invadidos por *R. pseudoacacia* que en suelos de ecosistemas dominados por *P. alba*, debido probablemente al elevado requerimiento energético de la fijación de N_2 y, por tanto, de P inorgánico para la producción de la molécula energética adenosín trifosfato (ATP) (Reinsvold y Pope 1987; Makoi y Ndakidemi 2008). Por tanto, *R. pseudoacacia* podría estimular la actividad PME para satisfacer sus necesidades de P (Izaguirre-

Mayoral et al. 2002). Apoyando esta idea, la menor concentración de P en las hojas senescentes de *R. pseudoacacia* que en las del resto de especies (*Capítulo 2 y 5*) indica una mayor removilización y uso de este nutriente por *R. pseudoacacia* para asumir el elevado coste energético de la fijación de N_2. La actividad PME se ha relacionado positivamente con la concentración de N y MO del suelo (Olander y Vitousek 2000; Makoi y Ndakidemi 2008; Nannipieri et al. 2011), por lo que las mayores concentraciones de N y MO en los suelos de *R. pseudoacacia* podrían favorecer dicha actividad enzimática. Del mismo modo, la menor actividad PME del suelo en ecosistemas invadidos por *A. altissima* respecto de ecosistemas dominados por *P. alba,* fue favorecida probablemente por la menor concentración de MO y N en los suelos de *A. altissima*. Por último, se ha reportado que la actividad PME y las tasas de mineralización del N en el suelo aumentan con la edad de la planta, por lo que un mayor tiempo de invasión de la especie exótica *R. pseudoacacia* puede aumentar su impacto sobre estas variables (Côté et al. 2000; Orczewska et al. 2012).

Impactos a nivel de comunidad y especie

A. altissima y *R. pseudoacacia* afectaron a la comunidad de bacterias edáficas y hongos acuáticos. Sin embargo, el efecto de la hojarasca de las EEI sobre las comunidades de macroinvertebrados acuáticos y sobre las especies de plantas del sotobosque no fue tan claro.

Los sitios invadidos por *A. altissima* presentaron diferente composición de la comunidad de bacterias edáficas que sitios nativos dominados por *P. alba,* (*Capítulo 3*). La estructura de la comunidad de bacterias del suelo de los sitios invadidos por *A. altissima* se correlacionó con menores tasas de nitrificación neta y mayor concentración de nitrato y relación NO_3^--N : NH_4^+-N. Esto puede ser debido a que los compuestos alelopáticos presentes en determinados tejidos de *A. altissima* (De Feo et al. 2003) podrían haber afectado a las bacterias nitrificantes del suelo, al igual que sucedió con la hojarasca de la especie *Elaeagnus angustifolia* L., la cual disminuyó la densidad y actividad de

microorganismos nitrificantes (Llinares et al. 1993). Los efectos producidos por *R. pseudoacacia* en la comunidad de bacterias del suelo no fueron tan marcados como los producidos por *A. altissima*, ya que fueron solo patentes en uno de los sitios invadidos. Los cambios producidos por *R. pseudoacacia* sobre la comunidad de bacterias del suelo se correlacionaron con una mayor actividad PME. En concordancia con estos resultados, otros estudios también han encontrado cambios en la comunidad de bacterias a la vez que aumentos en la actividad PME en suelos influenciados por el crecimiento de la especie herbácea *Zea mays* L. (Poaceae) (Kandeler et al. 2002). Se ha documentado que determinados cambios en las comunidades microbianas del suelo podrían favorecer a la especie invasora y afectar al crecimiento de las especies nativas (Niu et al. 2007; Grove et al. 2012; Xiao et al. 2014). Por ejemplo, la comunidad microbiana de los suelos procedentes de sitios invadidos por la especie *Ageratina adenophora* (Spreng.) R.M. King y H.Rob favoreció el crecimiento de la especie invasora e inhibió el crecimiento de las plantas nativas *Lolium perenne* L., *Eupatorium fortunei* Tuccz, y *Medicago sativa* L (Niu et al. 2007). Además, se ha reportado que los cambios en la comunidad microbiana pueden persistir varios años tras la eliminación de las especie invasoras *Cytisus scoparius* (L.) Link y *Berberis thunbergii* DC. (Elgersma et al. 2011; Grove et al. 2012, 2015), lo que podría dificultar las labores de restauración con especies nativas.

Los efectos alelopáticos de los árboles *A. altissima* y *R. pseudoacacia* sobre las comunidades de plantas del sotobosque, a través de extractos acuosos de hojas senescentes, no fueron mayores que los producidos por árboles nativos en sustrato papel, rechazándose en este caso la *Hipótesis de las Nuevas Armas* (HNA) para este mecanismo (Callaway y Ridenour 2004) (*Capítulo 4*). Nuestro estudio mostró que algunas especies de plantas del sotobosque podrían verse más afectadas por la alelopatía de ciertas especies de árboles. Por ejemplo, *R. pseudoacacia* podría afectar más a las otras especies de fabáceas testadas (*Trifolium repens* y *Medicago sativa*) y *A. altissima* a la especie *Lolium rigidum*. Estos resultados especie-específicos entre las especies donadoras de compuestos alelopáticos y las receptoras de los mismos (especies diana) llevan a pensar en

el establecimiento de diferentes comunidades de plantas del sotobosque en bosques dominados por cada una de las especies arbóreas. Sin embargo, otros factores como la modificación del microambiente por la hojarasca también influyen en la composición de las comunidades de plantas (Facelli y Picket 1991). Por tanto, la concordancia entre los resultados obtenidos en el laboratorio y los procedentes de los inventarios de vegetación podrían definitivamente confirmar el desplazamiento de determinadas especies del sotobosque por las especies arbóreas mediante la alelopatía (Fernández et al. 2013; Arroyo et al. 2015; Da Silva et al. 2015). El suelo de ribera mostró gran eficacia amortiguando los efectos alelopáticos encontrados en papel de germinación. Esto pudo deberse, en parte, a su elevada concentración de MO, la cual absorbe ácidos fenólicos inhibiendo por tanto su actividad alelopática (Pollock et al. 2009). Además, los microorganismos del suelo pueden también degradar los compuestos alelopáticos (Kobayashi 2004; Cipollini et al. 2012). En este sentido, la elevada actividad microbiana característica de los suelos de ribera (Schnürer et al. 1985; Naiman y Décamps 1997) pudo también determinar su elevada capacidad para reducir los efectos alelopáticos.

De acuerdo con la HNA (Callaway y Ridenour 2004), la hojarasca de las EEI, *A. altissima* y *R. pseudoacacia*, fué menos colonizada por hongos acuáticos que la de las especies nativas, *P. alba* y *F. angustifolia,* en el ecosistema fluvial (*Capítulo 5*). Por tanto, los hongos podrían no estar adaptados a los nuevos compuestos presentes en las EEI. Además, el menor efecto de los macroinvertebrados en las tasas de descomposición de las hojas senescentes de las EEI pudo atribuirse a dos razones no excluyentes: por un lado, los macroinvertebrados podrían no estar adaptados a los nuevos compuestos de las EEI según la HNA y, por otro lado los macroinvertebrados prefirieron comer hojas senescentes más colonizadas por hongos, es decir las nativas (Wright y Covich 2005). No se observaron efectos claros de la hojarasca de las EEI en la estructura de la comunidad de macroinvertebrados del ecosistema fluvial debido probablemente a que muchas especies usan la hojarasca y las bolsas de descomposición como refugio, para lo cual no influye la calidad de la hojarasca

o el origen exótico o nativo de la especie vegetal. Sin embargo, puede resaltarse una mayor tendencia de la familia Gammaridae a colonizar las hojas senescentes de la especie nativa *P. alba*. Esta asociación puede indicar una mayor preferencia de los Gammáridos por las hojas de *P. alba* y su composición específica de hongos. En este sentido, se ha constatado que, además del grado de colonización fúngica, la composición específica de la comunidad de hongos que colonizan las hojas senescentes es un factor relevante para la preferencia de los macroinvertebrados por determinadas especies de hojas (Arsuffi y Suberkropp 1989). Por tanto, el reemplazamiento de *P. alba* por las especies exóticas, *A. altissima* y *R. pseudoacacia,* en los ecosistemas de ribera podría afectar a la familia de macroinvertebrados acuáticos, Gammaridae.

Futuras líneas de investigación

De los trabajos realizados en esta Tesis surgen una serie de preguntas que quedan sin contestar y cuya respuesta ayudaría a aumentar el conocimiento de los impactos de las especies exóticas invasoras (EEI), *Ailanthus altissima* y *Robinia pseudoacacia,* así como tomar decisiones más ajustadas en cuanto a su gestión.

Relacionadas con los efectos en los ecosistemas

Sería de gran importancia, para la restauración de ecosistemas de ribera, conocer cuál es la capacidad que tienen las especies nativas para sobrevivir en suelos de ribera procedentes de ecosistemas invadidos por *A. altissima* y *R. pseudoacacia*. Si el crecimiento de especies nativas se viera inhibido, sería interesante distinguir qué características del suelo (nutrientes, comunidad microbiana, compuestos alelopáticos, etc.) son las responsables y cuál es el efecto de legado de las mismas. Al mismo tiempo es relevante conocer que especies nativas de plantas se establecen y crecen mejor en suelos invadidos por *A. altissima* y *R. pseudoacacia,* y por tanto cuáles son más aconsejables para la restauración de zonas de ribera tras la eliminación de las EEI. Por otro lado, sería conveniente analizar el riesgo de futuras invasiones, es decir, si los suelos modificados por *A. altissima* y *R. pseudoacacia* facilitan su propio crecimiento o el de otras EEI.

La realización de estudios en diferentes hábitats permitiría conocer cuáles son los factores ambientales que modifican los efectos de las EEI. Asimismo, sería interesante establecer si las variaciones inter-específicas de algunos rasgos de las EEI, como el contenido en nutrientes de las hojas, podrían modificar sus efectos en los ecosistemas. Además el estudio de los efectos a largo plazo desde la invasión ayudaría a conocer si se pierden las características propias de la especie invasora con el tiempo de invasión.

Relacionadas con las comunidades

Los estudios de preferencia de diferentes macroinvertebrados, tanto terrestres como acuáticos, por las hojas exóticas y nativas ayudarían a comprender mejor los impactos que las EEI producen en las comunidades de detritívoros. Además, en esta Tesis se ha demostrado que las especies invasoras producen impactos en las comunidades de bacterias del suelo pero se desconoce que grupos funcionales fueron afectados. También sería interesante comparar las comunidades de organismos acuáticos de ecosistemas fluviales influenciados por cada tipo de vegetación, exótica o nativa

Relacionadas con la alelopatía

Los estudios alelopáticos bajo condiciones naturales son actualmente un reto en ecología, ya que multitud de factores condicionan el efecto de los compuestos alelopáticos. Esta Tesis supone un primer paso en el estudio sobre los efectos potenciales de los extractos acuosos de hojas de EEI y nativas sobre las plantas del sotobosque en ecosistemas de ribera. Los estudios en campo utilizando por ejemplo carbono activo, el cual desactiva los compuestos alelopáticos en suelo, ayudarían a establecer la importancia de la alelopatía en condiciones naturales (ej. Gómez-Aparicio y Canham 2008a). La realización de estudios florísticos en ecosistemas invadidos por *A. altissima* y *R. pseudoacacia* y no invadidos (dominados por las especies nativas *F. angustifolia* y *P. alba*), ayudaría a interpretar los resultados obtenidos en el laboratorio, confirmando las posibles diferencias en las comunidades del sotobosque ocasionadas por los compuestos alelopáticos de los árboles exóticos y nativos. De acuerdo con el potencial alelopático de los árboles, se plantea ¿qué etapas del ciclo de las especies *A. altissima*, *R. pseudoacacia, F. angustifolia* y *P. alba* son más propensas a la producción de compuestos alelopáticos?, ¿cómo afecta el estrés de los árboles nativos e invasores a su potencial alelopático?. Por ejemplo, las hojas que caen de forma prematura (primavera tardía y verano) en bosques invadidos por *A.*

altissima y *R. pseudoacacia* tendrían mayor efecto alelopático que las hojas que caen en otoño?.

Además de por los lixiviados de la hojarasca, los compuestos alelopáticos pueden ser liberados por las plantas por otras vías, como los exudados de raíz, lo que contribuye a aumentar sus concentraciones en el suelo. En relación con esto, sería necesario saber cuáles son las concentraciones de compuestos alelopáticos presentes en los suelos del campo. Por otro lado, respecto de la reducción de la alelopatía en el suelo de ribera, documentada en esta Tesis, se plantean diversas cuestiones, como ¿cuáles son los componentes bióticos o abióticos del suelo que reducen los efectos alelopáticos?, ¿se acumulan o se degradan fácilmente los compuestos alelopáticos de las especies de estudio?, ¿la capacidad de reducción de la alelopatía varía en función del tipos de suelo?, ¿son los productos de degradación de algunos compuestos más eficaces contra el crecimiento de determinadas plantas?

Capítulo 8

Conclusiones generales/ General conclusions

Al ampliar el campo del conocimiento no hacemos sino aumentar el horizonte de la ignorancia.

Henry Miller

Fotografía: Cultivo en la ribera del arroyo Alboreca, cuenca alta del Río Henares (Guadalajara)
Por: Silvia Medina Villar

Photography: Alboreca stream, upper basin of the Henares River (Guadalajara)
By: Silvia Medina Villar

Conclusiones generales

Esta Tesis Doctoral evidencia que las especies exóticas invasoras *Ailanthus altissima* y *Robinia pseudoacacia* producen impactos ecológicos tanto a nivel de ecosistema, como de comunidad y especie, lo que supone una información relevante para la gestión de estas especies y la conservación y restauración de los ecosistemas de ribera que invaden.

A nivel de ecosistema

1. Los bosques de ribera invadidos por los árboles exóticos, *Ailanthus altissima* y *Robinia pseudoacacia*, producen similares cantidades anuales de hojarasca que los bosques dominados por las especies nativas de ribera, *Fraxinus angustifolia* y *Populus alba* (*Capítulo 2*).
2. La dinámica temporal de caída de hojarasca varía entre bosques invadidos por *A. altissima* y *R. pseudoacacia* y nativos dominados por *F. angustifolia* y *P. alba,* produciéndose picos adicionales de caída de hojarasca en primavera tardía y verano en los bosques invadidos (*Capítulo 2*)
3. La hojarasca de *A. altissima* y *R. pseudoacacia* presenta mayores concentraciones de nitrógeno (N) y fósforo (P) que la hojarasca de la especie nativa *P. alba* lo que probablemente determina mayores concentraciones de estos nutrientes en suelos bajo el dosel de los árboles exóticos invasores que en suelos bajo el dosel de *P. alba* (*Capítulo 2*).
4. Las características de la hojarasca de *A. altissima*, *R. pseudoacacia, F. angustifolia* y *P. alba* que mejor determinan su velocidad de descomposición en el ecosistema fluvial son la concentración de lignina, fibra ácido detergente y los ratios N:P, y lignina:P, que se correlacionan negativamente con las tasas de descomposición de la hojarasca, y la concentración de P, que se correlaciona positivamente con las mismas. De acuerdo con estas características, *A. altissima* aumenta las tasas de descomposición de la hojarasca en el ecosistema fluvial respecto de las especies nativas *F. angustifolia* y *P. alba,* mientras *R. pseudoacacia* las disminuye (*Capítulo 5*).

5. La especie fijadora de N_2, *R. pseudoacacia* aumenta la concentración de N mineral de suelos de ribera dominados por la especie nativa *P. alba.* Sin embargo, el efecto de *A. altísima* sobre el N total del suelo puede variar en función de las concentraciones pre-invasión de este nutriente, es decir, aumentando la concentración de N total cuando su concentración inicial es baja y viceversa (*Capítulo 2 y 3*).
6. La actividad fosfomonoesterasa y las tasas de mineralización de N del suelo son mayores en ecosistemas invadidos por *R. pseudoacacia* y menores en sitios invadidos por *A. altissima* que en ecosistemas dominados por la especie nativa *P. alba* (*Capítulo 3*).
7. En etapas iniciales de establecimiento, los árboles invasores *A. altissima* y *R. pseudoacacia* pueden alterar ciertas características del suelo, como el N total y el N mineral, respectivamente, pero necesitan más tiempo para producir impactos en otras características y procesos del ecosistema, como las tasas de mineralización o la comunidad de bacterias edáficas (*Capítulo 3*).

A nivel de comunidad y especie

8. La estructura de la comunidad de bacterias del suelo difiere claramente entre ecosistemas invadidos por *A. altissima* y ecosistemas dominados por la especie nativa *P. alba,* correlacionándose estas diferencias con la mayor concentración de nitrato y menores tasas de nitrificación en los suelos de *A. altissima.* Sin embargo, la estructura de la comunidad de bacterias de los suelos invadidos por *R. pseudoacacia* no difiere tan claramente de la de los suelos dominados por *P. alba,* correlacionándose las diferencias con la mayor actividad fosfomonoesterasa y concentración de amonio en los suelos de *R. pseudoacacia* (*Capítulo 3*).

9. En general, las especies exóticas invasoras, *A. altissima* y *R. pseudoacacia* no producen mayores efectos alelopáticos que las especies nativas sobre la germinación y crecimiento radicular de diversas especies de plantas del sotobosque. No obstante, *R. pseudoacacia* fue la especie más efectiva reduciendo el crecimiento de radícula de las especies de plantas del sotobosque en sustrato papel (*Capítulo 4*).
10. El suelo de ribera claramente amortigua los efectos alelopáticos observados en el papel de germinación, lo que sugiere la importancia de la consideración del suelo en estudios de alelopatía para evitar la sobreestimación de los efectos alelopáticos. También sugiere que la alelopatía debe no ser un mecanismo tan importante de interacción entre árboles y plantas del sotobosque en condiciones naturales (*Capítulo 4*).
11. Durante su descomposición en el ecosistema fluvial, las hojas senescentes de las especies exóticas, *A. altissima* y *R. pseudoacacia,* presentaron menor biomasa fúngica que las de las especies nativas, *F. angustifolia* y *P. alba*, debido probablemente a una menor adaptación de los hongos a los compuestos de las hojas exóticas, de acuerdo con la *Hipótesis de las Nuevas Armas* (*Capítulo 5*).
12. Los macroinvertebrados acuáticos mostraron un mayor efecto en la descomposición de la hojarasca nativa que en la descomposición de la exótica en el ecosistema fluvial, lo que puede deberse a que los macroinvertebrados prefieren consumir hojarascas más colonizadas por hongos y/o a que no están adaptados a consumir la hojarasca exótica, según la *Hipótesis de las Nuevas Armas* (*Capítulo 5*).

General Conclusions

This PhD Thesis evidences that the exotic invasive species *Ailanthus altissima* and *Robinia pseudoacacia* produce ecological impacts at different organization levels (ecosystem, community and species), which suppose a relevant information for the management of these species and the conservation and restoration of riparian ecosystems.

Ecosystem level

1. Riparian forests invaded by the exotic trees, *Ailanthus altissima* and *Robinia pseudoacacia*, produce similar quantities of annual litter as forests dominated by the native species, *Fraxinus angustifolia* and *Populus alba (Chapter 2*).
2. The temporal dynamic of litterfall varied between forest invaded by exotic trees, *A. altissima* and *R. pseudoacacia*, and forest dominated by native trees, *F. angustifolia* and *P. alba*. Specifically, additional peaks of litter (mainly leaves and flowers) were produced in late-spring and summer in invaded forests (*Chapter 2*).
3. Litter from exotic trees *A. altissima* and *R. pseudoacacia* showed greater concentrations of nitrogen (N) and phosphorus (P) than litter from the native tree *P. alba*, which likely determined the greater concentrations of these nutrients in the soil of forests invaded by the exotic species than in the soil of forests dominated by the native *P. alba* (*Chapter 2*).
4. The most suitable characteristics of leaf litter from *A. altissima*, *R. pseudoacacia, F. angustifolia* and *P. alba* that explain the low velocity of leaf litter decomposition are the concentration of lignin, acid detergent fiber, the N:P and lignin:P ratios. On the contrary, P concentration correlates positively with the leaf litter decomposition rates. According to these characteristics, *A. altissima* increase leaf litter decomposition rate in the fluvial ecosystem regarding native species, while *R. pseudoacacia* decrease it (*Chapter 5*).

5. The N_2-fixing species, *R. pseudoacacia*, is able to increase soil mineral N concentration of riparian forests dominated by the native species *P. alba*. However, the effect of *A. altisima* on soil total N can vary as a function of the pre-invasion or initial concentration of this nutrient in soil, i.e. increasing when initial concentrations of total N are low and decreasing when they are high (*Chapter 2 y 3*).
6. The soil phosphomonoesterase activity and N mineralization rate are greater in riparian ecosystems invaded by *R. pseudoacacia* and lower in those invaded by *A. altissima* than in native ecosystems dominated by the species *P. alba* (*Chapter 3*).
7. The exotic invasive tree species (*A. altissima* and *R. pseudoacacia*) can alter certain soil characteristics, such as total and mineral N, at early stages of establishment However, they need more time to produce impacts on other characteristics or ecosystem processes, such as soil bacterial communities or the mineralization rates (*Chapter 3*).

Community and species level

8. The structure of soil bacterial community clearly differ between riparian ecosystems invaded by *A. altissima* and those dominated by the native species *P. alba*, being the differences correlated to greater nitrate concentration and lower nitrification rates in *A. altissima* soils. However, the structure of soil bacterial community of ecosystems invaded by *R. pseudoacacia* did not clearly contrasts with that of the native ecosystems dominated by *P. alba*, being the slightly differences correlated to greater phosphomonoesterase activity and ammonium concentration in *R. pseudoacacia* soils *(Chapter 3*).

9. Generally, the exotic invasive species, *A. altissima* and *R. pseudoacacia*, did not produce greater allelopathic effects than native species on the germination and radicular growth of several undercanopy plant species. Nevertheless, *R. pseudoacacia* was the most effective species reducing the radicle growth of the undercanopy species using germination paper as substratum (*Chapter 4*).
10. Riparian soil clearly buffered the allelopathic effects observed using germination paper as substratum, which suggests the importance of considering the use of soil in allelopathic studies in order to avoid the overestimation of allelopathic effects. It also suggests that allelopathy could not be such an important mechanism of interaction between trees and understory species in natural conditions (*Chapter 4*).
11. During decomposition in a fluvial ecosystem, the senescent leaves of the exotic species, *A. altissima* and *R. pseudoacacia*, showed lower fungal biomass than those of the native species, *F. angustifolia* and *P. alba*, likely because of the lower adaptation of fungi to the exotic leaf litter compounds than to natives, according to *The Novel Weapons Hypothesis* (*Chapter 5*).
12. The aquatic macroinvertebrates showed greater effect on the decomposition of native litter than on the decomposition of exotic leaf litter in the fluvial ecosystem, which could be due to the macroinvertebrates prefer to consume leaf litter more colonized by fungi (i.e. native leaf litter) and/or because they are not adapted to consume exotic leaf litter, according to *The Novel Weapons Hypothesis* (*Chapter 5*).

References

La sabiduría consiste en saber cuál es el siguiente paso; la virtud, en llevarlo a cabo.

David Starr Jordan

Fotografía: Zona de la ribera del arroyo Alboreca, cuenca alta del Río Henares (Guadalajara)
Por: Silvia Medina Villar

Photography: Riparian zone of Alboreca stream, upper basin of the Henares River (Guadalajara)
By: Silvia Medina Villar

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Anexo

Todos los cambios, aun los más ansiados, llevan consigo cierta melancolía.

Anatole France

Fotografía: Plántulas de *Ailanthus altissima*
Por: Silvia Medina Villar

Photography: *Ailanthus altissima* seedlings
By: Silvia Medina Villar

Foto 1. **A**) Bosque invadido por la especie exótica *Robinia pseudoacacia,* **B**) bosque invadido por la especie exótica *Ailanthus altissima,* **C**) bosque dominado por la especie nativa *Fraxinus angustifolia* y **D**) bosque dominado por la especie nativa *Populus alba*. (**Capítulo 2**).

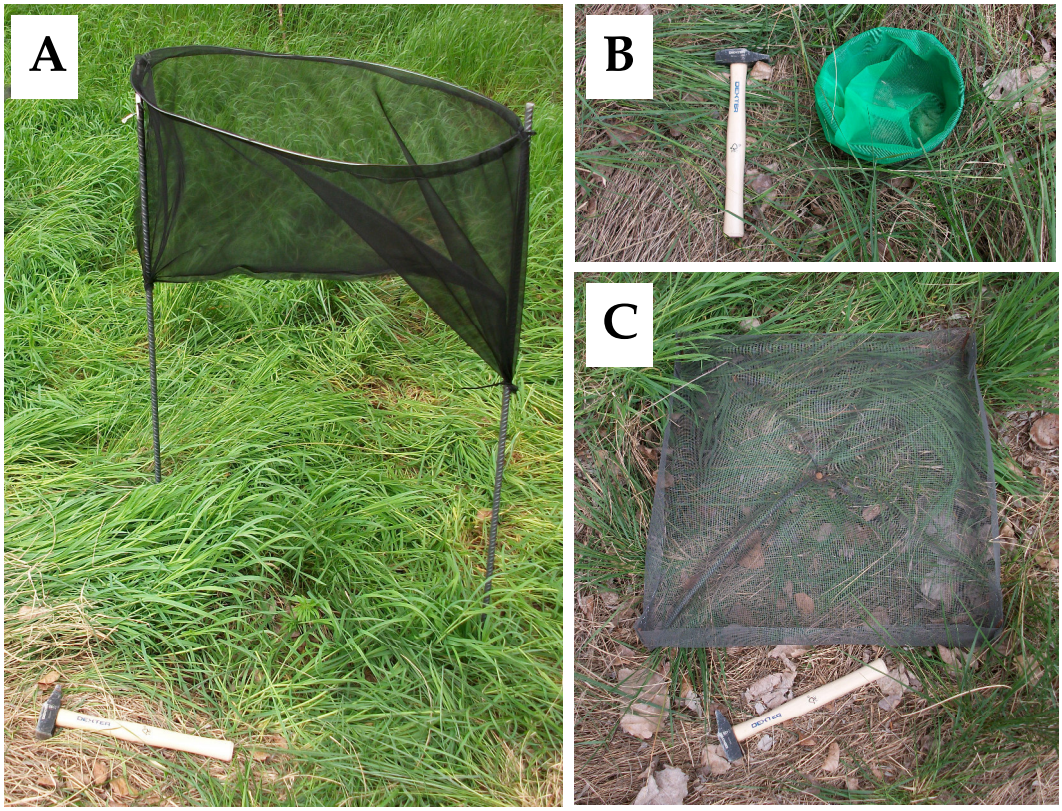

Foto 2. Trampas de recogida de hojarasca utilizadas en el **Capítulo 2**. **A**) "*Hanging trap*", **B**) "*Pot trap*" y **C**) "*Square trap*".

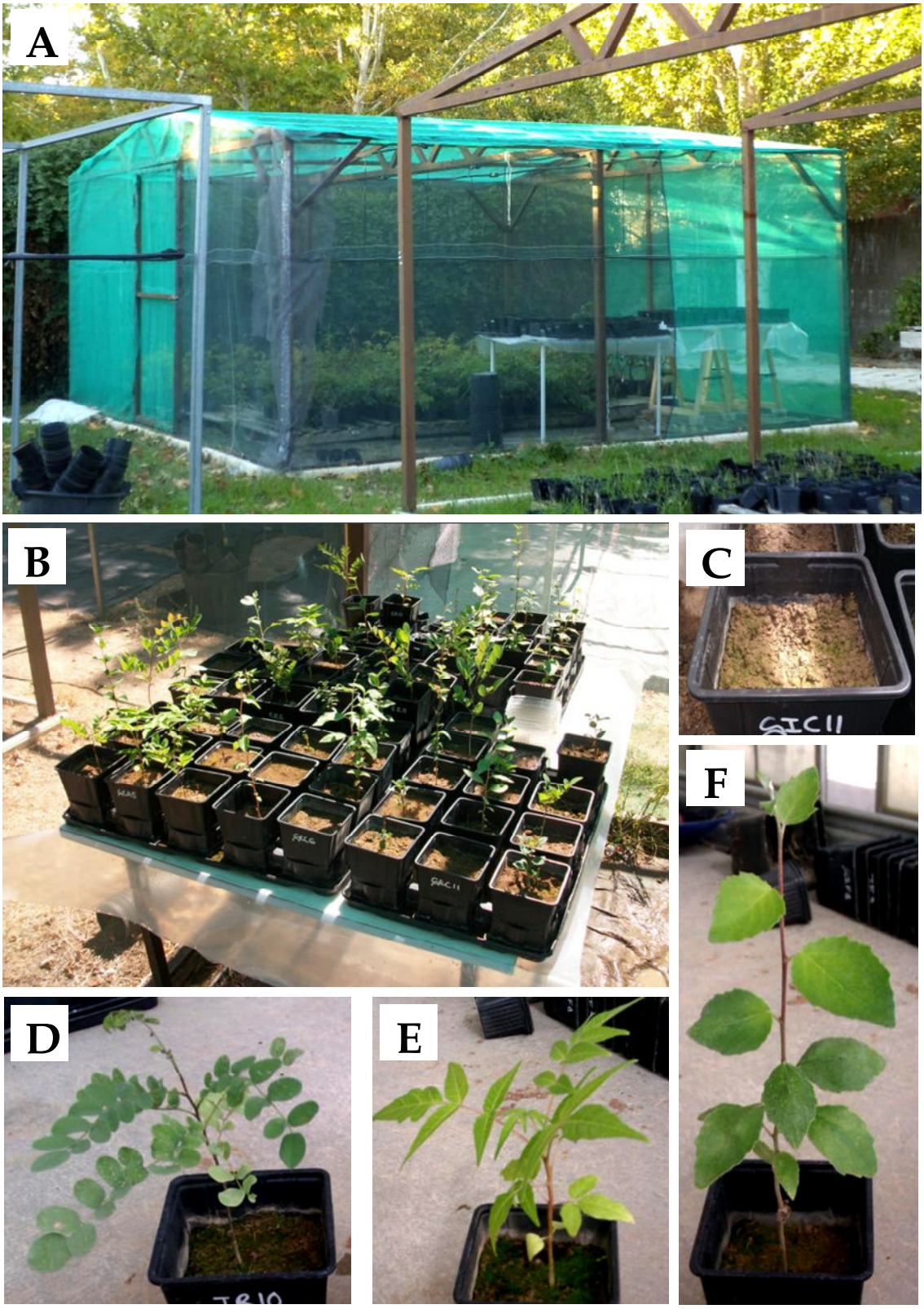

Foto 3. **A**) Parcela experimental con el 65 % de la luz solar total. **B**) Macetas con las plantas del experimento de invernadero (*Greenhouse experiment*). **C**) Maceta control, con suelo natural procedente de una zona de ribera dominada por la especie nativa *Populus alba.* **D**) Planta de seis meses de *Robinia pseudoacacia*. **E**) Planta de seis meses de *Ailanthus altissima* y **F**) planta de seis meses de *Populus alba.* (**Capítulo 3**).

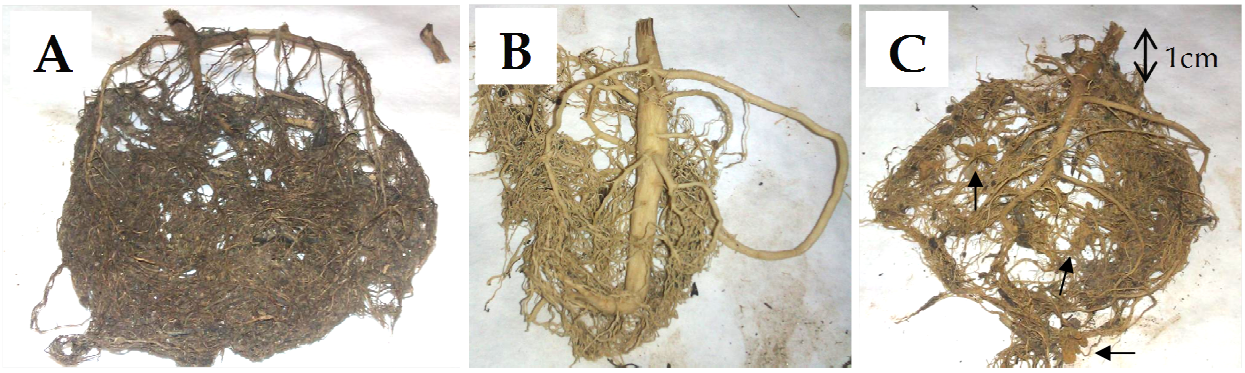

Foto 4. Raíces de las plantas de *Populus alba* (**A**), *Ailanthus altissima* (**B**) y *Robinia pseudoacacia* (**C**) después de seis meses desde la siembra. (**Capítulo 3**). Las flechas indican los nódulos de fijación de N_2 en las raíces de *Robinia pseudoacacia.*

Foto 5. Vegetación de ribera del sitio de estudio (Arroyo Alboreca, cuenca alta del Río Henares), caracterizada por la presencia de *Populus x euramericana.* (**Capítulo 5**).

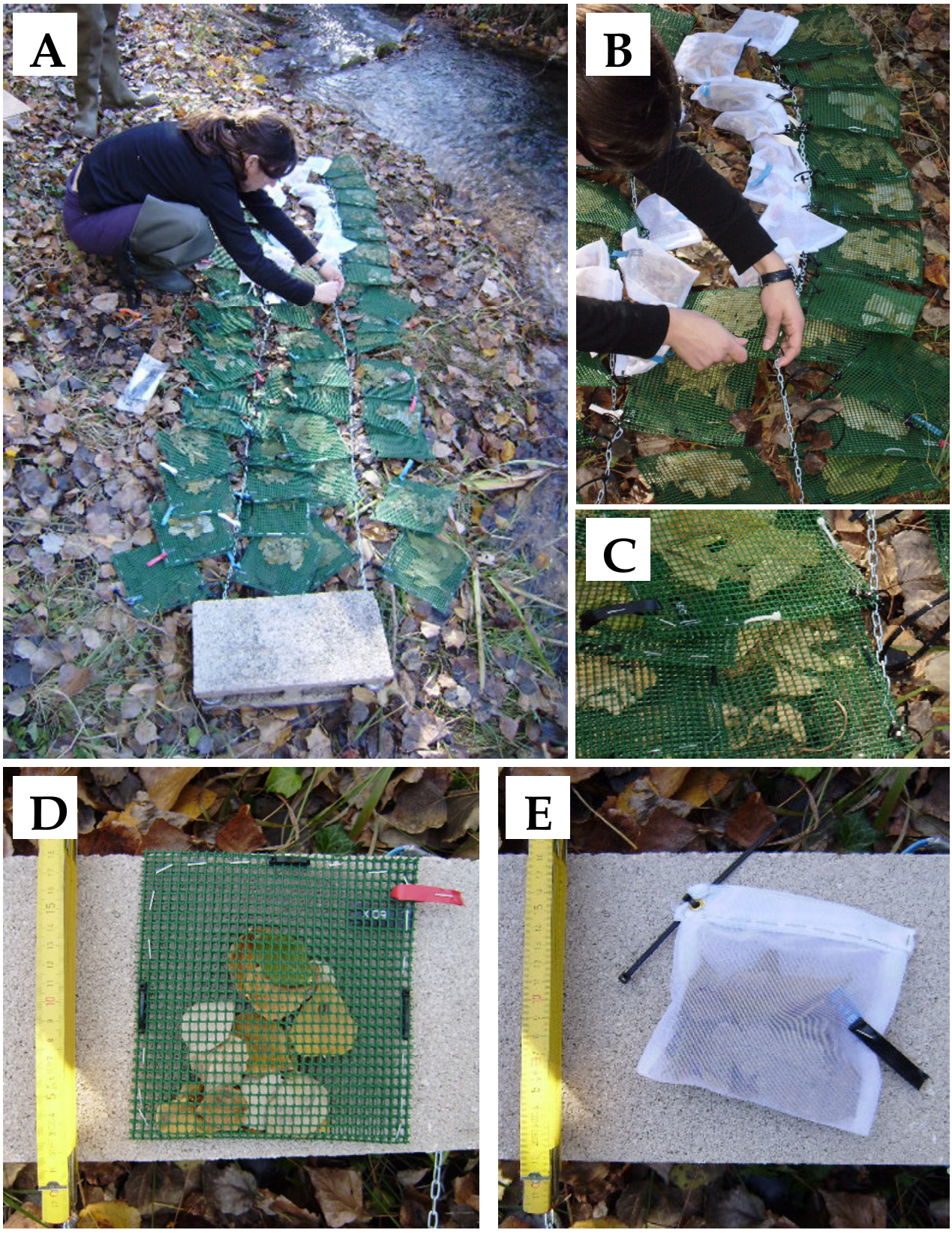

Foto 6. Disposición de las bolsas de descomposición para su introducción en el río (**A**, **B** y **C**). Bolsa de descomposición de luz de malla gruesa (*Coarse litter bag*) (**D**) y fina (*Fine litter bag*) (**E**). (**Capítulo 5**).

Foto 7. Disposición de las bolsas de descomposición en dos zonas de rápidos del Arroyo Alboreca (Cuenca Alta del Río Henares) (**Capítulo 5**).

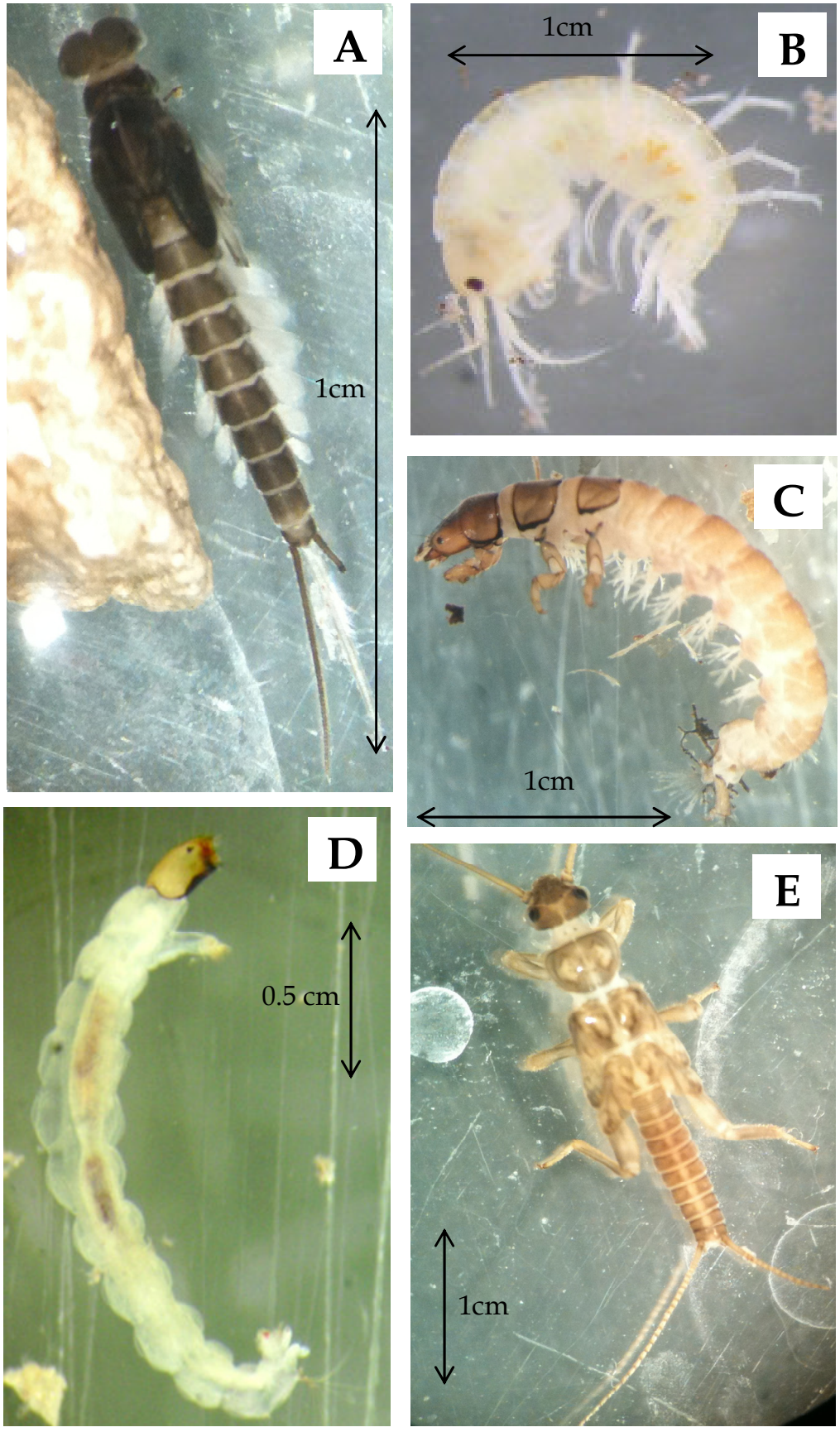

Foto 8. Diferentes familias de macroinvertebrados que colonizan las bolsas de descomposición. **A**) Baetidae (Orden Ephemeroptera). **B**) Gammaridae (Orden Amphipoda). **C**) Hydropsychidae (Orden Trichoptera). **D**) Chironomidae (Orden Diptera). **E**) Perlodidae (Plecoptera). (**Capítulo 5**).

Currículum Vitae

SILVIA MEDINA VILLAR
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Education

PhD. Student. Department of Life Sciences, Alcalá University, Spain (**2011 to Present)**
Thesis title: Ecological impacts of exotic invasive trees on the structure and functioning of fluvial and riparian ecosystems
PhD. Program: Ecology, Conservation and Ecosystem Restoration
Estimate defense data: July 2016

MSc. Renewable Energies (60 ECTS)**.** San Pablo CEU University, Spain **(2011)**
MSc Thesis title: Mixed photovoltaic solar-wind installation in a school center of Aguilar de Codes (Navarra)

BSc. Biology. Autonomous University of Madrid, Spain (**2009)**

Chemical Senior Technician of Analysis and Control. San Fernando HS, Spain (**2004)**

Award fellowships and grants

Research PhD fellow (FPI-MINECO) by the "Ministerio de Economía y Competitividad" Government of Spain, at Alcalá University **(2011-2015)**

Fellowships for Visiting Scholars in international research centers:

- **2012**. **Soil microbial community assessment by DNA analysis**. Centro de Ecologia Funcional, Universidade de Coímbra, Coímbra (Portugal). 4 months. Funds assigned: 6750 €
- **2013**. **Evaluation of litterfall process in invaded and native forest**. Department of Ecology and Evolutionary Biology. University of California, Santa Cruz, California (USA). 3 months. Funds assigned: 7400 €

Work experience

Research assistant at Alcalá University (2011-2015)

Technician at Department of Atomic Absorption and emission by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) in the *Investigation and Quality Control Centre* of the *National Institute of Consumption* of Spain (**3 months**) (**2004**)

Research experience

- *Participation in research projects*

1. **"Integral evaluation of exotic invasive trees over fluvial and riparian ecosystems of Castilla La Mancha** (POII10-0179-4700)". Alcalá University. Budget assigned: 80.000 € **(2010-2013)**

2. **"Assessing the invasive risk of exotic trees: distribution patterns invasive success and ecosystem impacts (INVARISK)"**. Alcalá University. Budget assigned: 83.490 € **(2011-2013)**

3. **"Program of I+D activities of Madrid about restoration of the natural environment". REMEDINAL-2** (S2009/AMB-1783) and **REMEDINAL-3** (S2013/MAE-2719). Madrid and European Union (FEDER y FSE funds) **(2010-2013)**

4. **"Quantification of the ecological impacts of riparian exotic trees on aquatic invertebrates".** Alcalá University. Budget assigned: 6000 € (**2013-2014)**

- *Analytical techniques used*

- Seed germination bioassays and measure of radicle length of several plant species
- Tests of the allelopathic potential of aqueous leaf litter extracts
- Acid digestion (Kjeldhal) of soil and vegetal material to determine the concentration of total N and P.
- Measure of the percentage of soil organic matter by soil ignition.
- DNA extraction from soil
- DNA amplification and DNA separation with PCR-DGGE (Plymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis) technique.
- NO_3^- and NH_4^+ extraction from soil, and microbial processes quantification (nitrification and ammonification)
- Leaf litter decomposition experiments in fluvial ecosystems.
- Identification of aquatic macroinvertebrate families colonizing leaf litter.

- *International experience*

2013 Evaluation of the litterfall process in invaded and native forests. Department of Ecology and Evolutionary Biology. University of California, Santa Cruz (USA) with Dra. Ingrid M Parker (**3 months**). The aim was to analyze data and write the manuscript of a paper which is published in the journal "Plant and Soil"

2012 Soil bacterial community assessment by DNA analyses. Center of Functional Ecology, Universidade de Coímbra (Portugal) with Dra. Susana Rodríguez Echeverría (**4 months**). The aim was to analyze soil bacterial community by means of molecular biology techniques, specifically PCR-DGGE technique

- *Reviewer of the following journals*

Acta Agronómica (2016)
Journal of Ecology (2016)
Journal of Ecology and the Natural Environment (2016)

Teaching experience

Lecturer in the following undergraduate subjects:

- Ecology (116h) Degree in Biology and Degree in Environmental Sciences
- Human Ecosystems (22h) Degree in Environmental Sciences
- Limnology (8h) Degree in Environmental Sciences
- Management and Conservation of Natural Resources (34h) Degree in Environmental Sciences
- Technics Applied to Field Work (28h) Degree in Environmental Sciences

Major advisor of a Senior thesis Degree in Biology. Project title: "Invasion and allelopathy: comparative study of the allelopathic effects of invasive (*Ailanthus altissima y Robinia pseudoacacia*) and native (*Fraxinus angustifolia* y *Populus alba*) species on *Bromus hordeaceus*". Mark: 8.9 (A+)

Publications

Peer reviewed publications

Medina-Villar S, Alonso A, Castro-Díez P, Pérez-Corona E**.** Allelopathic effects of exotic invasive and native trees on co-existing undercanopy species The soil as modulator. (Submitted to Plant and Soil)

Medina-Villar S, Rodríguez-Echeverría S, Lorenzo P, Alonso A, Pérez-Corona E, Castro-Díez P (2016) Impacts of the alien trees *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L. on soil nutrients and microbial communities**. Soil Biology and Biochemistry** 96: 65-73

Medina-Villar S, Castro-Díez P, Alonso A, Cabra-Rivas I, Parker IM, Pérez-Corona E (2015) Do the invasive trees, *Ailanthus altissima* and *Robinia pseudoacacia*, alter litterfall dynamics and soil properties of riparian ecosystems in Central Spain?. **Plant and Soil** 396: 311-324

Medina-Villar S, Alonso A, Rodríguez-Vázquez de Aldana B, Pérez-Corona E, Castro-Díez P (2015) Decomposition and biological colonization of native and exotic leaf litter in a Central Spain stream. **Limnetica** 34 (2): 293-310

Castro-Díez P, Alonso A, Gutiérrez López M, De las Heras-Puñal P, **Medina-Villar S**, Pérez-Corona E, Trigo Aza D, Rodríguez-Vázquez de Aldana B (2015) Integration of ecological impacts by invasive exotic plants: a methodological approach. **Ecosistemas** 24 (1): 12-17

Book chapters

Medina-Villar S, Pérez-Corona E, Alonso A, Castro-Díez P (In press) **Efectos alelopáticos de árboles exóticos invasores y nativos: ¿el suelo importa?** In: Ros G, Guerrero A, Hidalgo MA, Aguado J (Eds.) V Jornadas de Jóvenes Investigadores de la Universidad de Alcalá. Ciencias. Servicio de Publicaciones de la Universidad de Alcalá

Medina-Villar S, Alonso A, Pérez-Corona E, Castro-Díez P (**2013**) **Impacto de las especies exóticas en los ecosistemas de ribera.** In: Ros G, Guerrero A, Aguado J, Hidalgo MA (Eds.) IV Jornadas de Jóvenes Investigadores de la Universidad de Alcalá. Servicio de Publicaciones de la Universidad de Alcalá

Medina-Villar S, Alonso A, Castro-Díez P (**2011**) **Las invasiones de plantas exóticas de ribera: impactos sobre los ecosistemas fluviales**. In: Aguado J, Blanco M, Ros G, Hidalgo MA (Eds.) III Jornadas de Jóvenes Investigadores de la Universidad de Alcalá. Servicio de Publicaciones de la Universidad de Alcalá

Congress contributions

Oral communications

- International

Medina-Villar S, Pérez-Corona E, Alonso A, Castro-Díez P. **Allelopathic effects of invasive and native trees on herbaceous species: the role of soil as modulator.** 4º Congreso Ibérico de Ecología. Coimbra, 16-19 June 2015

Medina-Villar S. Litter production in riparian invaded and non invaded patches in a river of Central Spain. Lab meeting at Department of Ecology and Evolutionary Biology. University of California, Santa Cruz (USA)**,** December, 2013

- National

Medina Villar S, Rodríguez-Echeverría S, Lorenzo P, Alonso A, Pérez-Corona E, Castro-Díez P. "**Impactos de los árboles exóticos invasores, *Ailanthus altissima* y *Robinia pseudoacacia*, en los nutrientes y la comunidad de bacterias del suelo de un ecosistema de ribera".** I *Simposio sobre Interacciones Planta-Suelo*. ICA-CSIC, Madrid, 25-26 February 2016

Medina-Villar S, Pérez-Corona E, Alonso A, Castro-Díez P. "**Efectos alelopáticos de árboles exóticos invasores y nativos: ¿El suelo importa?".** *V Jornadas de Jóvenes Investigadores de la Universidad de Alcalá*. Alcalá de Henares, 1-3 December 2014

Medina-Villar. "Descomposición y colonización biológica de hojarasca exótica en un arroyo". Lab meeting at the Department of life sciences, Alcalá University, April 2014

Medina-Villar S, Alonso A, Pérez-Corona E, Castro-Díez P. "**Impacto de las especies exóticas en los ecosistemas de ribera".** *IV Jornadas de Jóvenes Investigadores de la Universidad de Alcalá*. Alcalá de Henares, 28-30 November 2012

Medina-Villar S, Alonso A, Castro-Díez P**. "Las invasiones de plantas exóticas de ribera: impactos sobre los ecosistemas fluviales".** *III Jornadas de Jóvenes Investigadores de la Universidad de Alcalá*. Alcalá de Henares, Madrid, 1-3 December 2010

Poster communications

- International

Castro-Díez P, Alonso A, Gutiérrez-López M, De las Heras Puñal P, **Medina-Villar S**, Pérez-Corona E, Trigo Aza D, Rodríguez Vázquez de Aldana B. **Integration of ecological impacts by invasive exotic plants: a methodological approach.** *4º Congreso Ibérico de Ecología*. Coimbra, 16-19 June 2015

Medina-Villar S, Castro-Díez P, Alonso A, Cabra-Rivas I, Valle-Torres G, Parker IM, Pérez-Corona E. **Impacts of the exotic trees *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacia* L. on litterfall patterns and soil nutrients in riparian forests of Central Spain.** 4th International Symposium on Weeds and Invasive Plants. Montpellier, 19-23 May 2014

Medina-Villar S, Alonso A, Pérez-Corona E, Rodríguez-Echeverría S, González-Muñoz N, Valle-Torres G, Castro-Díez P. **Effects of invasive trees (*Ailanthus altissima* and *Robinia pseudoacacia*) on nutrients and enzymatic activity of a riparian soil.** 7th European Conference on Invasive Alien Species. NEOBIOTA. Pontevedra, 12-14 September 2012

Medina-Villar S, Langa M, Alonso A, Castro-Díez P. **Assessing the impact of exotic trees leaf litter on the macroinvertebrate community in an upper reach of Henares River (Guadalajara, Spain).** Symposium for European Freshwater Sciences. Girona, 27 June-1 July 2011

- National

Medina-Villar S, Alonso A, Rodríguez-Vázquez de Aldana B, Castro-Díez P, Pérez-Corona E. "**Efectos de dos especies exóticas invasoras (*Ailanthus altissima* y *Robinia pseudoacacia*) sobre el ciclo de nutrientes y la biomasa fúngica de un ecosistema fluvial".** EEI 2012, *4º Congreso Nacional sobre Especies Exóticas Invasoras*. Pontevedra, 10-11 September 2012

Medina-Villar S, Alonso A, Rodríguez-Echeverría S, Pérez-Corona E, Castro-Díez P. "**Evaluación del impacto del crecimiento de árboles exóticos sobre la comunidad microbiana de un suelo nativo"**. *Terceras Jornadas Técnicas de los Jardines Botánicos: hacia la sostenibilidad*. Alcalá de Henares, 6-8 June 2012

Additional skills

Languages

- **Spanish** native
- **English** Advanced level
  - English course. (9 months). British Council, Alcalá University, Madrid (2012)
  - English course (3 weeks) Londonderry (Northern Ireland) (2010)
  - English course (6 weeks) Portsmouth (Britain) (2009)
- **Portuguese** Advanced level

Software

- Microsoft Office: Word, Excel, Powerpoint
- Statistic programs: SPSS, R Package, Community Analysis Package (CAP), JMP, CANOCO, Prisma

Scientific curses attended

- Simulation models in Ecology (16h). Alcalá University, Madrid (2014)
- Brief introduction to R: Data handling, graphs and regression (9h). Autonomous University of Madrid (2011)
- Advance Introduction to R lenguage (12h). Polytechnic University of Madrid (2011)

Academic Societies

Asociación Española de Ecología Terrestre (AEET)